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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Uncaria tomentosa* (Willd. ex Schult.) DC., cortex

Final

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Uncaria tomentosa*, cortex
Herbal preparation(s)	
Pharmaceutical form(s)	
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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Different definitions regarding the plant part used can be found in literature:

According to the WHO monograph Uncariae cortex consists of the dried stem bark of *Uncaria tomentosa* (Willd.) DC. (Rubiaceae) (WHO monograph 2007).

According to the definition of USP, cat's claw consists of the inner bark of the stems of *Uncaria tomentosa* (Willd.) DC. (Rubiaceae). It contains ≥ 0.3% pentacyclic oxindole alkaloids as isopteropodine, calculated on the dried basis, as the sum of speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine and isopteropodine. The tetracyclic alkaloids content is ≤ 0.05% (USP 37).

According to Hagers Handbuch the herbal substance is the root bark (Blaschek *et al.* 1998).

Uncaria tomentosa is a scrambling liana, up to 20-30 meters long, main stem up to 25 cm in diameter. It grows as high as altitudes of 500-600 meters above sea level, in high forests with abundant insolation and reaches 18-19 m in height (Roth and Lindorf, 2002; WHO, 2007). The plant, also known as the "life-giving vine of Peru", "cat's claw", "saventaro", or "uña de gato", is a thick woody vine indigenous to the Amazon rain forest and other tropical areas of South and Central America. The plant has hook-like thorns, growing largely along the vine in a leafy pattern, which resembles the claws of a cat.

The vernacular names 'cat's claw' and 'uña de gato' in Spanish may lead to confusion because they are used for several plant species in tropical America.

From the genus Uncaria the two most common species used and marketed interchangeably for their various properties are *Uncaria tomentosa* and Uncaria guianensis. *Uncaria tomentosa* is evidently the preferred species partly because of its higher alkaloid content (Erowele and Kalejaiye, 2009).

Main constituents

Major classes of compounds (**Table 1**) identified in *Uncaria tomentosa* include oxindole and indole alkaloids (0.15-4.60%), pyroquinovic acid glycosides, organic acids, proanthocyanidins, sterols, and polyoxygenated triterpenes (WHO, 2007; Gonzales and Valerio, 2006).

Table 1: Constituents identified from *Uncaria tomentosa* (all plant parts) (Gonzales and Valerio, 2006)

Oxindole alkaloids	Pentacyclic: Formosanine (uncarine B), Pteropodine (uncarine C), Isopteropodine (uncarine E), Speciophylline (uncarine D), Speciophylline N-oxide, Uncarine F N-oxide, Mitraphylline, Isomitraphylline. Tetracyclic: Rhynchophylline, Rhynchophylline N-oxide, Isorhynchophylline, Isorhynchophylline N-oxide, Rotundifoline, Isorotundifoline, Corynoxeine, Isocorynoxeine

Indole alkaloids	Pentacyclic: Akuammigine, Tetrahydroalstonine, Isoajimalicine. Tetracyclic: Hirsutine, Hirsutine N-oxide, Dihydrocorynantheine, Hirsuteine, Corynantheine
Quinovic acid glycosides	Approximately 9 compounds (e.g. quinovic acid (28-1)-b-D-glucopyranosyl ester
Pyroquinovic acid glycosides	Tomentoside A, Tomentoside B
Organic acids	Oleanolic acid
Proanthocyanidines	(-)-Epicatechin, cinchonain 1a, cinchonain 1b
Sterols	β-Sitosterol, stigmasterol, campesterol
Triterpenes	Ursolic acid derivatives, oleanan-type triterpenes, cincholic acid glycosides

About 50 different components have been isolated from *Uncaria tomentosa* (considering all plant parts), 35 of which have been identified in only a couple of other species (Heitzman *et al.* 2005). There are three classes of compounds that are thought to play an important role in the activity of cat's claw. These compounds are the alkaloids, quinovic acid glycosides and polyhydroxylated triterpenes (Valerio and Gonzales,. 2005).

Quinovic acid glycosides having a C-3, a C-27, a C-28, a C-3, -28 or a C-3, 27 glycosylation patterns are characteristic to the family Rubiaceae (Aquino *et al.* 1991, Aquino 1997). Tomentoside A and B were the first reported naturally occurring pyroquinovic acid glycosides (Kitajima *et al.* 2003), hence these compounds can be considered as markers of the species.

The alkaloid content can vary 10- to 40-fold depending on cultivation techniques and the season when the plant is harvested (Kemper, 1999; Laus and Keplinger, 1994). *Uncaria tomentosa* occurs in two chemotypes, each varies greatly in its alkaloid content. One predominantly contains pentacyclic oxindole alkaloids, while the other is rich in the tetracyclic oxindoles. The alkaloid composition of *Uncaria tomentosa* is changing significantly over time period and plant generations. Laus and Keplinger reported that the total alkaloid content varied from 0.036 to 3.83% (w/w) of the dried root bark of *Uncaria tomentosa*, depending on harvestings; and individual plants switched from one alkaloid pattern to the other over time (Laus and Keplinger, 1994). Based on the study of Wurm *et al.* (1998), it has been stated, that the mixture of the two chemotypes of cat's claw are unsuitable for therapeutic use, unless certified to contain less than 0.02% tetracyclic oxindole alkaloids (Laus and Keplinger, 1997; Barnes *et al.* 2002).

- Herbal preparation(s)

Herbal preparations obtained from the stem bark:

Powdered herbal substance (according to WHO): It consists of finely broken pieces of wood, bast and bark, and clear, crystalline particles of dried sap (WHO, 2007).

Powdered Cat's Claw (according to USP 37) is cat's claw reduced to a powder or very fine powder. It contains no less than 0.3% of pentacyclic oxindole alkaloids, calculated on the dried basis, as the sum of speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, and isopteropodine (USP 37)

Powdered Cat's Claw Extract (according to USP 37) is prepared from Cat's Claw by extraction with hydroalcoholic mixtures or other suitable solvents. The ratio of plant material to extract is between 4:1

and 6:1. It contains no less than 90% and not more than 110% of the labelled amount of pentacyclic oxindole alkaloids, calculated on the dried basis, as the sum of speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, and isopteropodine. It may contain suitable added substances (USP 37).

Cat's Claw Capsules (according to USP 37) contain Powdered Cat's Claw Extract. Capsules contain no less than 90% and not more than 110% of the labelled amount of Powdered Extract, calculated as pentacyclic oxindole alkaloids (USP, 37).

Cat's Claw Tablets (according to USP 37) contain Powdered Cat's Claw Extract. Tablets contain no less than 90% and no more than 110% of the labelled amount of Powdered Extract, calculated as pentacyclic oxindole alkaloids (USP 37).

There is a Brazilian product, 50 mg/g herbarium gel cream which contains 50 mg *Uncaria tomentosa* bark extract/g cream. The 50 mg extract is equivalent of 0.03 to 0.045 mg of the oxindole alkaloids (no more details regarding extraction solvent and DER available)(Caldas *et al.* 2010).

Herbal preparations obtained from the root bark:

PTI-00703: proprietary extract of the root or the inner bark of *Uncaria tomentosa* (Cummings *et al.* 2000, Quinn *et al.* 2004, Snow *et al.* 2000). **Herein after referred to as 'a proprietary extract of the root or the inner bark of Uncaria tomentosa** (PE) '.

There is another Brazilian product (tablets) containing *Uncaria tomentosa* extract. The extract was prepared by ultra-turrax extraction from ground bark using 70% ethanol. The HPLC analysis of the dry extract presents 2.57% pentacyclic oxindole alkaloids (POAs) content, which was calculated with reference to external calibration curves of mitraphylline. The extract analysis showed absence of tetracyclic oxindole alkaloids in the sample (Araújo M *et al.* 2012, Farias *et al.* 2011).

Herbal preparations lacking information regarding the plant part used:

Batch-2: **aqueous extract** from of *Uncaria tomentosa* (Shi *et al.* 2010). **Herein after referred to as a 'novel aqueous extract from of *Uncaria tomentosa*** (NAE)**'.**

There is a commercially available, patented water-soluble filtered *Uncaria tomentosa* aqueous extract, standardised to 8–10% CAEs (carboxy alkyl esthers) and almost free from oxindole alkaloids (≤0.05%)**.** It is produced from heating 150 g of bark in 5 l of tap water for 18-24 hours at 95 °C, until the hot water extract is concentrated to about 900-1000 milliliters by evaporation. The dark brown extract then adjusted to exactly 1000 millilitres. After decanting the soluble fraction and ultra-filtrating the resulting water extract to remove all components>10,000 MW and larger, it is dried according to U.S. Patent 6,039,949. Quantification of the extract was performed by Sheng *et al.* (2000b). The amount of alkaloids in 100 g: Uncarine F (2.39 mg), speciophylline (13.75 mg), mitraphylline (4.34 mg), isomitraphylline (1.73 mg), pteropodine (20.17 mg), isopteropodine (5.96 mg). It can be found in several food supplements. **Herein after referred to as 'a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE)' (Åkesson *et al.* 2003a, Guthrie *et al.* 2011, Lamm *et al.*, 2001, Mammone *et al.* 2006, Sheng *et al.*, 1998, 2000a, 2000b, 2001, 2005).

Herbal preparations from the entire root:

Twenty mg dry extract from *Uncariae tomentosae radix* / capsule (DER 8-12:1, extraction solvent acidified water) 14.7 mg/g POA, no TOA**.** Contains 0.93% pteropodine, 0.53% speciophylline, 0.34% mitraphylline, 0.25% isopteropodine, 0.16% Uncarine F and 0.05% isomitraphylline (Winkler *et al.* 2004). **Herein after referred to as '**(HE)'.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable

1.2. Search and assessment methodology

The assessment of Uncariae tomentsae cortex is based on the following literature:

- Articles and references retrieved from databases (PubMed, ToxNet, SciFinder) or internet sources (e.g. Google, Google scholar) until March 2014. The databases were searched using the terms [*Uncaria tomentosa*], [Una de gato] and [cat's claw].
- Monographs on *Uncaria tomentosa*: Hagers Handbuch (Blaschek, 1998), WHO (2007).
- Handbooks, textbooks and review articles were also used.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 2: Overview of data obtained from marketed medicinal products containing stem bark or root

Active substance	Indication	Pharmaceutical form	Regulatory Status
Stem bark			
Powdered herbal substance	Relief of minor articular pain associated to inflammatory processes	Capsules 150 mg twice a day	Traditional use 1998 Spain
Powdered herbal substance		Capsules 380 mg four times daily	Traditional use 2000 Spain
Powdered herbal substance Tablet		Tablets 500 mg three times daily	Traditional use 2002 Spain
Powdered herbal substance	To relieve the symptoms of disorders of the locomotor system As adjuvant therapy to enhance the protective capacity of the body	150 mg/capsule Adults an adolescent: 300 mg three times daily Children between the age of 3-12: Up to 150 mg three times daily	Healing product 2000 Hungary
Powdered herbal substance	To relieve the symptoms of inflammation of locomotor disorders as adjunct to medicinal and physiotherapeutic treatment As adjuvant therapy to enhance the protective capacity of the body	330 mg/capsule Adults an adolescent: 330-660 mg three times daily Children between the age of 3-12: 330 mg once a day	Healing product 2001 Hungary

Active substance	Indication	Pharmaceutical form	Regulatory Status
Uncariae tomentosae corticis ethanolic ext. sicc. (DER: 8:1): 100 mg [with 5 mg pentacyclic oxindole alkaloid (POA) content at least] Extraction solvent ethanolum 80%	For the treatment of disorders of the locomotor system – rheumatism, inflammation of the joints, arthritis- alone or as adjuvant of medicinal or physiotherapeutic treatment. For strengthening the immuno-system, for improvement of protective power of the body, as an adjuvant therapy of medicinal treatment in acute and recurrent infectious diseases	100 mg/tablet Adults: Generally 100 mg daily, up to 100 mg three times daily Children above 12 years of age: according to recommendation of the therapist	Healing product 2001 Hungary
20 mg Uncariae tomentosae corticis ethanolic ext. sicc. (15:1) [with 5mg pentacyclic-oxindol alcaloide (POA) content at least]/g extraction solvent : ethanolum 80%	Acute and chronic inflammation of the skin. For cuts, bruises to promote epithelisation. Alleviation of inflammation in case of burn (I. grade) solar erythema, insect bite.	Apply the gel in thin layer to the affected area of the skin 2-3 times daily	Healing product 2006 Hungary
Uncariae tomentosae corticis sine pericarpii pulvis with 0.2% total alkaloid content expressed in mitrofilline at least and up to 0.07% tetracyclic oxindole alkaloid content	For the treatment of disorders of the locomotor system - rheumatism, inflammation of the joints, arthritis-alone or as adjuvant of medicinal or physiotherapeutic treatment. For strengthening the immuno-system, for improvement of protective power of the body, as an adjuvant therapy of medicinal treatment in acute and recurrent infectious diseases.	Tea 700.0 mg/tea bag Adolescent: tea infusion prepared from 700 mg-1400 mg three times daily. Maximum daily doses: 2100 mg three times Children above 12 years of age: tea prepared from 700 mg twice a day 700 mg should be poured with 2-2.5 dl of boiling water, to keep covered for 10 minutes In case of use of 1400 mg or 2100 mg the quantity of water should be increased for 6-7 dl.	Healing product 2001 Hungary
Root			
Dry extract (entire root is extracted, not the bark separately), DER 8-12:1, extraction solvent acidified water. Minimum 13 mg/g pentacyclic oxindole alkaloids, maximum 0.5 mg/g tetracyclic oxindole alkaloids	Adjuvant therapy to an antirheumatic standard therapy in patients with rheumatoid arthritis.	20 mg/capsule Oral; adults: 3 x 20 mg daily. Duration of use: onset of clinical efficacy within 3-4 months. Clinical trials provide evidence of safe use over a period of 12 months.	Full marketing authorisation May 2000, withdrawn 2015 Austria

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable

Information on other products marketed in the EU/EEA (where relevant)

Food-supplements in the literature:

- The commercially available filtered aqueous extract from the bark of *Uncaria tomentosa* (AE)' (Åkesson *et al.* 2003a, Guthrie *et al.* 2011, Lamm *et al.*, 2001, Mammone *et al.* 2006, Sheng *et al.*, 1998, 2000a, 2000b, 2001, 2005) can be found in several food supplements.
- 150 mg *Uncaria tomentosa* dry extract/capsule containing product (Castaňeda *et al.* 1998).

2.1.2. Information on products on the market outside the EU/EEA

Uncaria tomentosa is considered a dietary supplement by the US Food and Drug Administration. In the USA *Uncaria tomentosa* is available in 300 mg capsules to be taken three times daily, 1000 mg time-release capsules to be taken once daily, liquid concentrate (8:1 in a 20% alcohol) to be diluted in water and taking 1 to 3 times daily, and bark to be used for tea (No more information regarding the herbal preparations available; Cupp *et al.*, 2000).

There is a Brazilian product, 50 mg/g herbarium gel cream which contains 50 mg *Uncaria tomentosa* bark extract/g cream. The 50 mg extract is an equivalent of 0.03 to 0.045 mg of the oxindole alkaloids (No more information regarding the herbal preparations available) (Caldas *et al.* 2010).

There is another Brazilian product (tablets) containing 100 mg of dry *Uncaria tomentosa* extract. The *Uncaria tomentosa* extract was prepared by extraction from ground bark using 70% ethanol (Araújo M *et al.* 2012, Farias *et al*. 2011).Two products are reported from Peru: one of them contains *Uncaria tomentosa* hydroalcoholic, (80% of ethanol) spray-dried extract (drug extract ratio 8:1) with 5.61% of total oxindole alkaloids, the other an aqueous freeze-dried extract with a total 0.26% oxindol alkaloids (Aguliar *et al.* 2002).

2.2. Information on documented medicinal use and historical data from literature

Peruvian tribes (longest recorded history of use: Asháninka indians) have used cat's claw (*Uncaria tomentosa*) as an anti-inflammatory agent, contraceptive, emmenagogue; for cancer, gastric ulcer, diarrhoea, asthma, wounds, gonorrhoea, arthritis, rheumatism, acne, diabetes, diseases of the urinary tract, menstrual irregularity; to recover from childbirth, but also as a tonic to ward off disease or as abortifacient. Sometimes cat's claw is used in combination with other local herbs (such as chuchuhuasi (*Maytenus krukovii*) bark) to treat arthritis. It has been traditionally contraindicated in pregnancy, during lactation and for children. A related species (*Uncaria guianensis*) has been used in South America for wound healing, as a sedative and to treat intestinal ailments, but is not considered as strong as *Uncaria tomentosa.* Cat's claw has been used in Peru and Europe since the early 1990s as an adjunctive treatment for cancer and AIDS, as well as other diseases targeting the immunological system (Kemper, 1999; Taylor, 2002).

Uncaria tomentosa was first described in 1830 and first studied in Peru by the German biologist Brell in 1950 (Cabieses, 1994). Scientific studies with cat's claw began in the early 1970s when Klaus Keplinger from Austria organised the first work on *Uncaria tomentosa*. Keplinger's work in the 1970s and 1980s led to several extracts of cat's claw being sold in Austria and Germany as well as four US patents describing extraction procedures for a group of chemicals, oxindole alkaloids and the immunostimulating actions of these alkaloids found in *Uncaria tomentosa*. These novel oxindole alkaloids fuelled research and business worldwide. Hence, the presence of *Uncaria tomentosa* has declined in Peruvian rainforests by overharvesting. The lower growing and easier to find *Uncaria guianensis* is thus a common adulterant in many cat's claw products.

Referring to an article in Suriname Bulletin (1962) Hagers Handbuch (Blaschek, 1998) mentions that the root bark and the stem bark as well has been used in Peru in the form of aqueous or alcoholic infusion for arthritis, gastritis and other disturbances of the gastro-intestinal system, treatment of cancer and for different skin disorders.

According to Roth and Lindorf (2002) traditionally in Peru two spoonfuls of the bark (plant part not specified) of *Uncaria tomentosa* are boiled in 1.5 l water for 30 minutes and left to cool. Half a glass of this liquid is taken three times a day before meals.

In Peru the root bark is applied as decoction (20 g sliced material in 1 l water for 45 minutes). The liquid is decanted and losses due to evaporisation are replenished. This bitter decoction is said to be a 10-day dose. From HPLC analyses using a previously published method (Laus and Keplinger, 1994) of similarly prepared decoctions the daily dose was estimated at 4 mg oxindole alkaloids (Keplinger *et al.* 1999).

According to Cabieses, *Uncaria tomentosa* was traditionally used in massive concentrations as a contraceptive and in lower concentrations to dissolve tumours, but not as an abortive. It is said, that women in Campa tribe boil six kilograms of the root in one litre of water, until it is reduced to about 250 cc. They filter it and drink the fluid during the menstrual period for three consecutive months in order to avoid pregnancy for three or four years. It is said that to dissolve tumours, the dosage is much less, 0.5 kilogram root in 5 litres of water for 30 minutes (Cabieses, 1994).

Table 3: Overview of historical data

Herbal preparation	**Documented Use / Traditional Use**	**Pharmaceutical form**	**Reference**
Root, root bark, stem bark and leaves	Traditionally to treat gonorrhoea, dysentery, arthritis, rheumatism, gastric ulcers and various tumours, reputed to be contraceptive	Commercial products (tablets, capsules) contain varying amounts of material, ranging from 25 to 300 mg stand-ardised extract and from 400 mg to 5 g of plant material	Barnes J *et al.* 2002
Root bark or stem bark	Arthritis, gastritis or other disturbance in the gastro-intestinal system, treatment of cancer and for different skin disorders	In Peru in the form of aqueous or alcoholic infusion 20 g sliced material in 1 l water for 45 minutes as a decoction	Blaschek W *et al.* 1998
Vine bark (not specified), root	As an immune stimulant and an adjunctive therapy for cancer (to reduce side effects of chemotherapy and protect cells) as a bowel cleanser and anti-inflammatory for Crohn's, colitis, diverticulitis, irritable bowel syndrome, and other bowel problems as an anti-inflammatory for arthritis (all kinds) and muscle pains/strains/injuries as a general daily tonic (to tone, balance, and strengthen all body functions) for stomach ulcers and ulcerative colitis and as an ulcer preventative/ stomach and bowel protector)	Decoction: 1 cup twice daily Powder: 1-2 g 2-3 times daily Fluid Extract: 2-4 ml twice daily Tincture: 2-4 ml twice daily	Rain-tree tropical database 2004
Dried stem bark	Uses described in pharmacopoeias and well-established documents: symptomatic treatment of arthritis, rheumatism and gastric ulcers. Uses described in traditional medicine: Treatment of abscesses, asthma, fevers, urinary tract infection, viral infections and wounds. As emmenagouge. The efficacy of *Uncaria* extract was examined in patients with immunodeficiency (cancer, HIV),	Average daily dose: Extracts, 20.0-350.0 mg. Capsules and tablets: 300.0-500.0 mg, one capsule or tablet two to three times. Herpes simplex labialis increased mutagenic potential (smoker), rheumatoid arthritis	WHO monograph 2007
Root	Traditional use: “Dissolve” tumours	Decoction (0.5 kg drug + 5 l water, boiling for 30 minutes), *per os* One cup of decoction three times a day	Cabieses 1994 Peru
Root	Traditional use: Contraception	Decoction (5-6 kg drug+1 l water, boiling until 250 cc) During menstruation for three consecutive months, *per os*	Cabieses 1994 Peru

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form	Reference
Bark	Traditional use: prostatitis, anti-inflammatory, tumours, AIDS, rheumatism, diabetes, arthritis, contraceptive	Decoction, *per os*	Taylor 2002 Peru
Bark	Traditional use: Anti-inflammatory	Infusion, *per os*	Klinar *et al.* 1995 Peru
Vine	Traditional use: Gonorrhoea, dysentery	*Per os*, form not stated	Taylor 2002 Colombia
Root bark	Traditional use: cancer, arthritis	Decoction, *per os*	Taylor 2002 Peru
Infusion of the root bark of *Uncaria tomentosa*	Traditional use: cancer, arthritis, intestinal disorders	Infusion, *per os*	Taylor 2002 Peru
Bark of *U. tomentosa*	Traditional use: for asthma, inflammations of the urinary tract, fevers, abscesses, haemorrhages, impurities of the skin, irregularity of the menstrual cycle, immune disorders, AIDS, cancer, bone pain, gastric ulcers, urinary tract cancer in women, cirrhosis, gastritis, to recover from childbirth, normalise the body and cleanse the system, as a kidney and body cleanser, antacid, cellular reconstituent, cicatrizant	*Per os*, form not stated	Taylor 2002 Peru
Decoction of the bark of *U. tomentosa*	Traditional use: malignant tumour, rheumatism, arthritis, diabetes, cirrhosis of the liver	Two spoonfuls of the bark of *U. tomentosa* are boiled in 1.5 l water for 30 minutes and left to cool. Half a glass of this liquid is taken three times a day before meals	Roth and Lindorf 2002

2.3. Overall conclusions on medicinal use

There are two countries in the European Union where products containing *Uncaria tomentosa* (Willd.) DC, cortex have been used traditionally for medicinal purposes:

In Spain preparations containing powdered stem bark of *Uncaria tomentosa* have been in medicinal use since 1998. In Hungary the powdered stem bark of *Uncaria tomentosa* and an ethanolic extract have been marketed since 2000 and 2001. Although preparations containing pulvered stem bark of *Uncaria tomentosa* have been in medicinal use for a period of at least 15 years in the European Union, evidence for the medicinal use for at least 30 years outside of the European Union of the plant part used, the herbal preparation, indication and posology is not available as requested by Directive 2004/24/EC. Consequently, a European Union herbal monograph on *Uncaria tomentosa* (Willd.) DC, cortex cannot be established at present.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Although, the evidence for the medicinal use for at least 30 years outside of the European Union is not fulfilled in the case of *Uncaria tomentosa*, cortex, there are two countries where preparations containing powdered stem bark of *Uncaria tomentosa* are fulfilling the medicinal use for a period of at least 15 years in the European Union requested by Directive 2004/24/EC, hence the preclinical studies linked to the indications (articular pain, rheumatism, for strengthening the immuno-system) of these products were taken into account as primary pharmacodynamics. In the secondary pharmadynamics

there are some experiments that served as starting points for the clinical investigations (e.g Alzheimer's disease)

3.1.1. Primary pharmacodynamics

Herbal Preparations

Anti-inflammatory properties

In vitro

Bark (not specified)

The administration of an **aqueous extract of the dried bark** of *Uncaria tomentosa* (AUT) (100 µg/ml) attenuated ($p<0.05$) peroxynitrite-induced (300µM) apoptosis in HT29 (epithelial) and RAW 264.7 cells (macrophage). The extract at concentrations 50-200 µg/ml inhibited the LPS (1 µg/ml) induced iNOS gene expression in HT29 cells and nitrite formation ($p<0.05$) in both cell-lines at 100 µg/ml concentration. In RAW 264.7 cells, LPS decreased cell viability from 95.9% to 80.1%, AUT (100 µg/ml) increased it to 87% ($p<0.05$). In RAW 264.7 cells the simultaneous administration of AUT and LPS caused a significant, 60% inhibition of nitrite formation, $p<0.05$. AUT (100 µg/ml) also inhibited the activation of NF-κB in RAW 264.7 cells in the presence of LPS. The extract was prepared with boiling water (20 g/L) for 30 minutes and then was left overnight in room temperature then it was filtered and diluted to 5 mg/ml (Sandoval-Chachón *et al.* 1998).

Sandoval *et al.* (2000) reported that an **aqueous extract of the micropulverised bark** of *Uncaria tomentosa* (AMU) with or without concentration by freeze-drying have a protective effect against LPS induced TNF-α production in murine macrophages (RAW 264.7 cells). LPS (0.5 µg/ml) increased TNF-α levels from 3 to 97 ng/ml. AMU suppressed TNF-α production by approximately 65-85% ($p<0.01$), but at concentrations considerably lower than its antioxidant activity: freeze-dried EC_{50}=1.2 ng/ml, micropulverised EC_{50}=28 ng/ml. The aqueous decoction of the micropulverised bark was made by boiling water (20 g/L) for 30 minutes, and then filtered (Sandoval *et al.* 2000).

The anti-inflammatory effects of an **aqueous extract of the bark** of *Uncaria tomentosa* (AT) and *Uncaria guianensis* (AG) were compared. Anti-inflammatory activity was assessed *in vitro* by inhibition of TNF-α and nitrite production from RAW 264.7 cells, exposed to LPS (50 ng/ml). The total oxindole and pentacyclic alkaloid content of AT was 35 fold > AG. The IC_{50} value for inhibition of TNF-α production was significantly ($p<0.01$) higher for AT (14.1 ng/ml) vs. AG (9.5 ng/ml) with maximum inhibitions of 70.6% and 75.5%, respectively. These concentrations were considerably lower than that required for antioxidant activity. The extracts were prepared with boiling water, 50 g/l w/v for 30 minutes, then filtered and freeze-dried. For the experiments they were dissolved in water (20 mg/ml) (Sandoval *et al.* 2002).

The anti-inflammatory activity of two commercially available *Uncaria tomentosa* **bark extracts, a spray-dried** extract (**extraction solvent ethanol 80%,** DER 8:1), containing 5.61% of total oxindole alkaloids (TOA) and **aqueous freeze-dried** *Uncaria tomentosa* extract, TOA 0.26% were assessed at the concentration range of 50-500 µg/ml by NF-kB EMSA and at 50 µg/ml by COX-1 and -2 assay. Activation of NF-kB was nearly completely reduced by pre-treatment of Jurkat T cells with 500 µg/ml of the hydroalcoholic extract, while the aqueous extract only slightly prevented NF-kB DNA binding at this concentration. Hydroalcoholic extract exhibited an inhibition of COX-1 and -2 of 7.8% and of 21.7%, respectively. In contrast, an inhibitory activity of 32.7% was measured for COX-1 and of 12.2% for COX-2 for the aqueous freeze-dried extract (Aguliar *et al.* 2002).

Stem bark

Piscoya *et al.* (2001) reported that *Uncaria tomentosa* and *guianensis* (commercially available purified freeze-dried extracts of **stem bark**) inhibited TNF-α synthesis/release on a murine macrophage cell line stimulated by LPS. Following low concentrations (1–1000 ng/ml or 50 ng/ml) of pre-treatment with the freeze-dried aqueous extracts of the two species, a significant and dose-dependent reduction in TNF-α levels after LPS stimulation were observed. The inhibition of TNF-α was approximately equivalent *for Uncaria tomentosa* and *Uncaria guaianensis* with low IC_{50} values (10.2, 10.9 ng/ml, respectively) and with a maximum inhibition of 79% and 73%, respectively. However, the extracts had no effect on unstimulated PGE2 production but did significantly reduce PGE2 production stimulated by LPS, suggesting inhibition of cyclooxygenase 2 (COX-2) expression. The dose required (10 µg/ml) was greater than that for suppression of TNF-α production.

In vivo

Bark (not specified)

An **aqueous extract of the bark** of *Uncaria tomentosa* (AUT) was administered orally in drinking water (5 mg/L) to Sprague-Dawley rats (n=3/group) with indomethacin induced intestinal inflammation. The chronic model of intestinal inflammation was induced by two sc. injections of indomethacin (7.5 mg/kg). Animals were divided into four groups: vehicle control group, injected with indomethacin, injected with indomethacin and supplemented with AUT, injected vehicle and supplemented with AUT. The extract markedly attenuated indomethacin enteritis as evident by reduced myeloperoxidase activity ($p<0.05$), morphometric damage and metallothionein expression (69.6 and 216.6 µg/ml with and without AUT, $p<0.05$). The extract was prepared with boiling water (20 g/L) for 30 minutes and then was left overnight in room temperature, filtered and diluted to 5 mg/ml (Sandoval-Chachón *et al.* 1998).

Cisneros *et al.* (2005) investigated the effect of an **aqueous decoction of the bark** of *Uncaria tomentosa* (ADU) on ozone inhalation caused acute pneumonitis, characterised by a high number of infiltrating neutrophils (PMNs) immediately after exposure and increased levels of protein in BALF (bronchoalveolar lavage fluid) in mice. 96 mice were randomly assigned into three groups (n=32), one received water, the other two groups ADU extract (DER 1:14) (50% and 100%) diluted with distilled water *ad libitum* for 8 days. After the treatment they were exposed to 3 ppm O^3 for 4 hours and killed 0 or 8 hours after it. When compared to untreated controls, ADU-treated mice had significantly ($p<0.05$) lower levels of protein in BALF, lower degree of epithelial necrosis, higher number of intact epithelial cell nuclei in bronchial wall, and decreased number of PMNs in the bronchiolar lumen. The lung tissue protective effect was dose related. The aqueous decoction was prepared by boiling dry *Uncaria tomentosa* bark (20 g/L) in deionised water for three hours; approximately 280 ml of extract were obtained from each 20 g of dry bark, yielding about 14 ml of aqueous extract per gram of dry bark.

The anti-inflammatory activity of two *Uncaria tomentosa* **bark extracts,** a **spray-dried** extract (extraction solvent ethanol 80%, DER 8:1), containing 5.61% of total oxindole alkaloids (TOA) and **aqueous freeze-dried** *Uncaria tomentosa* extract (TOA 0.26%) were assessed and compared in carrageenan-induced paw oedema model in BALB/c mice. The extracts dose-dependently and significantly decreased the carrageenan-induced increase in paw volume as compared with control rats. The two extracts were diluted in 1 ml distilled water in concentrations 500, 200, 100 and 50 mg/kg and were administered through a gastric probe during 8 days of pre-treatment. The paw inflammation was measured 4 hours after the carrageenan injection. The negative control group received distilled water, the positive control group indomethacin (7 mg/kg). Hydroalcoholic extract (50 mg/kg) produced an anti-inflammatory effect similar to 7 mg/kg of the non-steroidal drug

indomethacin, while the aqueous freeze-dried extract exhibited the same effect at 200 mg/kg. Both extracts used were commercially available products (Aguliar *et al.* 2002).

The anti-inflammatory effects of oral treatment for 3 days with **micropulverised *Uncaria tomentosa* bark** 5 mg/ml, given in drinking water prior to the oral administration of indomethacin, protected against indomethacin-induced gastritis (20 mg/kg bodyweight) in Sprague-Dawley rats (number not given). *Uncaria tomentosa* elicited a protective effect ($p<0.01$), the degree of gastric mucosal injury was markedly attenuated and prevented TNF-α mRNA expression and apoptosis (Sandoval *et al.* 2002).

Root bark

Aquino *et al.* (1991) reported, that the $CHCl_3$/MeOH (9:1) (50 mg/kg) and the water fractions (84 mg/kg) of a **petroleum ether extract of the root bark** of *Uncaria tomentosa*, were the most active using the carrageenan-induced edema model in rat (male Wistar Nossan) paw. The oral pre-treatment with the fractions caused 69.2 and 41.2% inhibition at 3 hours of the inflammatory response, respectively. The anti-inflammatory effects of the MeOH and $CHCl_3$ fractions were not significant. Subjects (n=5/group) received either indomethacin (5 mg/kg), vehicle or fractions at doses equivalent to 2 g of dry bark/kg. One hour after drug administration 1% carrageenan was injected and the paw volumes were measured hourly for 5 hours.

A significant inhibition of stress-induced ulcer formation in rats was shown following pre-treatment with 3 ml of a **decoction** of *Uncaria tomentosa* **root bark** added to drinking water (no further details are discussed). The size and number of large gastric ulcers were significantly inhibited compared to controls (McKenna *et al.* 2002).

Root

An **aqueous extract** of the root of *Uncaria tomentosa* was able to inhibit the oedema induced by *Bothrops asper* snake venom. Six Sprague-Dawley rats were pre-treated with 250 or 500 mg/kg extract *i.p.*, then one hour later 100, 50, 25, 10 and 5 µg/50 µl of *B. asper* venom was injected in one leg, NaCl to the other. Control groups received dexamethasone and diphenhydramine. The extract of *Uncaria tomentosa* diminished considerably the oedema-forming activity of the venom. The doses of 250 mg/kg and 500 mg/kg showed an anti-inflammatory effect at all times ($p<0.05$), except for the dose of 250 mg/kg at one hour. Inhibition (paw volume) was observed at 1, 2, 4, 6 and 24 hours after venom injection. The root was extracted with 70°C water (10% w/v) for 30 minutes, then filtered, evaporated and freeze-dried (Badilla *et al.* 2006).

Unknown plant part

Intestinal morphology of indomethacin-treated rats was fully restored and liver metallothionein expression and inflammatory indices were supressed by *Uncaria tomentosa* (details regarding plant **part and extract missing,** conference abstract). *In vitro* experiments indicated that the anti-inflammatory action of *Uncaria tomentosa* is primarily mediated through the inhibition of inflammatory gene expression involving a suppression of NF-κB. *Uncaria tomentosa* was only effective when administered prophylactically, consistent with its effects on gene expression (Miller *et al.* 1999).

Abe *et al.* (2002) examined the effect of oral administration of *Uncaria tomentosa* **70% ethanolic extract** (paper in Japanese, plant **part not known)** (0.125, 0.5, 2 g/kg) on carragenan-induced oedema in normal and in prednisolone treated mice. The extract was given 1, 23 and 25 hours after the carragenan injection. The extract did not affect inflammatory response, just when it was administered in combination with the extract of *Harpagophytum procumbens* (0.8 g/kg), it significantly ($p<0.05$) lowered the thickness of the edema. The *Harpagophytum procumbens* extract alone did not have any effect on the inflammation either.

Immunmodulatory, immunostimulant properties

In vitro

Bark (not specified)

The acute effects of *Uncaria tomentosa* (**70% ethanolic extract of the bark**, 2.57% pentacyclic oxindole alkaloids) on granulocyte-macrophage colony forming cells (CFU-GM) were reported. Colony-forming cell (CFC) assays were performed with human hematopoietic stem/precursor cells obtained from umbilical cord blood. An *in vitro* CFC assay showed an increase in CFU-GM size and mixed colonies (CFU-GEMM) size at the final concentrations of 100 and 200 µg/ml. The spray-dried extract was prepared by ultra-turrax extraction (Farias *et al.* 2011).

Allen-Hall *et al.* (2007) showed that treatment of THP-1 monocyte-like cells with a **95% ethanolic extract of the bark** of *Uncaria tomentosa* (6:1) inhibited the MAP-kinase signalling pathway and altered cytokine expression. The treatment with 1 or 10 µl *Uncaria tomentosa* extracts augmented LPS-dependent expression of IL-1β by 2.4-fold, while inhibiting the LPS-dependent expression of TNF-α by 5.5-fold ($p<0.05$). It is highly unusual to see the response of these two cytokines to operate in opposite directions. The treatment of THP-1 monocytes with *Uncaria tomentosa* extracts alone augmented the expression of IL-1β by greater than 20-fold, but did not affect the expression of TNF-α ($p<0.05$). The treatment of LPS-stimulated THP-1 cells with *Uncaria tomentosa* extracts blocked ERK1/2 and MEK1/2 phosphorylation in a dose-dependent manner.

Allen-Hall *et al.* (2010) reported that a **95% ethanolic extract** of the bark of *Uncaria tomentosa* inhibited the LPS-dependent activation of specific NF-κB and AP-1 components. TNF-α and IL-1ß are usually regulated similarly and share a number of common promoter elements, including NF-κB and AP-1. Treatment with *Uncaria tomentosa* inhibited the secretion of TNF-α in LPS-treated THP-1 cells in a dose-dependent manner: 10-320 µg/ml *Uncaria tomentosa* extract inhibited TNF-α secretion by 33-95%. In contrast, treatment with *Uncaria tomentosa* enhanced LPS-dependent expression of IL-1β: 40 µg/ml enhanced IL-1β by 1.2-fold and 160 µg/ml enhanced IL-1β by 1.4-fold, although 320 µg/ml *Uncaria tomentosa* completely blocked LPS-dependent secretion of IL-1β. The ability of *Uncaria tomentosa* to inhibit TNF-α production was diminished when NF-κB activation was prevented, while IL-1β expression was unchanged. The bark was extracted by exhaustive percolation with 95% ethanol.

Stem bark

Two commercial aqueous extracts of the stem bark of different collections of *Uncaria tomentosa* were studied on alveolar macrophages. The commercial extracts derived from different areas of Peru, one of the plant materials for the extract (AAT-6.98 mg/g total oxindole alkaloid content) was collected from the Asháninka region of Peru**.** The extract was prepared by **water extraction of the bark** and lyophilisation of the extract. The other water extract (MUT-5.57 mg/g total oxindole alkaloid content) was prepared by water extraction and atomisation of material derived from lowland tropical collections between Cusco and Manu National Park. Both of them contained uncarine, speciophylline, mitraphylline, isomitraphylline, pteropodine, and isopteropodine. The extracts were tested at 0.025-0.5 mg/ml. MUT greatly stimulated IL-1 (10X at max. effect) and IL-6 (7.5X at max. effect) production by rat macrophages in a dose dependent manner in the range of 0.025–0.1 mg/ml compared to control. They were also able to enhance IL-1 (5.2X) and IL-6 (<2X) in LPS-stimulated macrophages compared to control. AAT had comparable trends in results (not specified) (Lemaire *et al.* 1999).

Reis *et al.* (2008) assessed the immunoregulatory activity of a **50% ethanolic extract** of **the stem bark** of *Uncaria tomentosa* in an *in vitro* DENV (*Dengue* Virus-2) infection model. No significant alterations were detected with the treatment of hydro-ethanolic extract in the measured TNF-α, IL-6,

IL-10 and IFN-α levels. IL-6, which is strikingly induced after monocyte infection, apparently was not inhibited neither by *Uncaria tomentosa* nor dexamethasone (reference) treatments.

One hundred and seventy-eight **plant extracts prepared with ethanol 70%** (for 48 hours 25°C) from the pharmacopoeia of the Tacana, an ethnic group from Bolivia, were screened for immunmodulatory activity using complement cascade inhibition and ADP-induced platelet aggregation inhibition assays. Six extracts impaired both complement pathways (classical-CPW and alternative-APW), amongst them **the stem bark of *Uncaria tomentosa*** (UT). The IC_{50} values for CPW and APW were 124 and 151 µg/ml respectively. Positive control, heparin had $IC_{50:}$ 74 and 558 µg/ml. Concentrations were tested from 250 to 3.9 µg/ml. Anti-inflammatory activity did not rely on COX inhibition. UT extract was not an effective inhibitor (at 5 mg/ml) of ADP-induced platelet aggregation, which is linked with the inhibition of the COX pathway (Deharo *et al.* 2004).

Åkesson *et al.* (2003a) confirmed that **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) inhibits proliferation of normal mouse T and B lymphocytes and that the inhibition is not caused by toxicity or by induction of apoptosis. AE was tested in concentrations from 0.25-1 mg/ml in different assays. AE efficiently inhibited proliferation of primary lymphocytes, stimulated with either T or B cell mitogens (Con A, CD-3 or LPS). The inhibition was more prominent in cultures (mouse spleen cells) stimulated with T cell mitogens as compared with the B cell mitogen LPS, where higher concentrations of AE were required to reach the same level of inhibition. Furthermore, the extract did neither interfere with IL-2 production nor IL-2 receptor signalling (mouse CTLL-2 cells). Since there was no discrete cell cycle block in AE -treated cells, the authors proposed that retarded cell cycle progression caused the inhibition of proliferation. Moreover, AE treatment reduced NF-κB activity with almost 50% (LPS stimulated 70Z/3 cells).

Root and stem bark

In a granulocytic test, the average increase in phagocytic activity shown by the **stem bark** was found to be 10% to 20%, while that of the **root bark** was on average 30% to 40% (extract type not discussed). The difference in potency was attributed to the low alkaloid content of stem bark and to differences in patterns of the oxindole alkaloids present (McKenna *et al.* 2002).

Unknown part

Using FACS-based assays, Holderness *et al.* (2007) screened primary bovine cells for novel γδ T cell agonists. γδ T cells are innate immune cells that participate in host responses against pathogens and cancers. *Uncaria tomentosa* (commercial product) was extracted in room temperature with **water** (10 ml/g) then lyophilised to determine the approximate dry weight: 17.6 mg/ml. The extract (44 µg/ml) induced IL-2Rα expression on γδ T cells after 48 hours in culture and induced activation and proliferation of the cells as well, as detected in a 5-day CFSE assay. Activity of the extract was specific to γδ T cells and furthermore, and the activity was not limited to one γδ T cell subset. Positive control agonists were LPS and Con A. Subsequent analysis demonstrated that γδ T cell agonist activity of *Uncaria tomentosa* extract was due to the condensed tannin fractions of the drug. Polyphenols contributed to 56% of *Uncaria tomentosa* and 63.7% of the polyphenols were tannins.

The immunmodulatory effect of two commercially available extracts (HE) of the root of *Uncaria tomentosa* (**HCl and 96% ethanolic**) were investigated in human peripheral mononuclear cells (PBMC) stimulated with the PHA and Con A *in vitro*. Compared to unstimulated cells, PHA (phytohaemagglutinin) and Con A (concanavalin A) increased the production of neopterin and the degradation of tryptophan ($p<0.01$). The extracts inhibited both effects in a dose dependant manner; the lowest effective concentrations were 500-1000 µg/ml ($p<0.05$). With the highest concentrations complete suppression of mitogen-induced neopterin production and tryptophan degradation was observed, indicating, that extracts interfere with immunopathogenetic pathways, which involve the Th-

1 type cytokine IFN-γ. **The HCl extract** contained 0.93% pteropodine, 0.53% speciophylline, 0.34% mitraphylline, 0.25% isopteropodine, 0.16% Uncarine F and 0.05% isomitraphylline. **The 96% ethanolic extract contained** 0.73% isopteropodine, 0.28% pteropodine, 0.17% isomitraphylline, 0.13% mitraphylline, 0.05% Uncarine F, 0.04% speciophylline, 0.015% rhynchophylline, 0.009% isorhynchophylline, 0.003% corynoxeine and 0.001% isocorynoxeine. A concentration range of 500-4000 µg/ml was applied to the cultures from the extracts (Winkler *et al.* 2004).

In vivo

Bark (not specified)

Bednarek *et al.* (2002) investigated the influence of the grounded **bark** of *Uncaria tomentosa* (commercial product*)* on the course of an experimentally induced local pneumonia in calves. The study consisted two groups, Group I (n=10, 3600 mg/calf/day of *Uncaria tomentosa*) and Group II (n=10, placebo), treated for 17 days, orally with the drug in the form of 200 ml **decoction**. Inflammatory inducer (mineral oil) was used twice on the 4th day. The body temperatures were significantly higher at several time points ($P<0.05$; $P<0.01$) in the group of the untreated calves (40.5-41^{o}C), compared with the treated one (39.0-40.5^{o}C). During the treatment, the total number of WBC and the total numbers and percentages of mid-size cells and neutrophils dropped considerably in the group of calves treated with *Uncaria tomentosa*, in comparison with the untreated group, but the number of lymphocytes was significantly ($p\leq0.05$) higher in the treated animals at the end of the treatment ($10.1x10^9$/L), in contrast to those values at the beginning of the treatment ($8.6x10^9$/L). In the treated group, the total number of $CD2^+$ cells (T lymphocytes) and the percentage of $CD4^+$ cells (T helper lymphocytes) were significantly higher at the end of the treatment ($7.9x10^9$/L, 40.3%, respectively, $p\leq0.05$), as compared with respective values initially ($5.7x10^9$/L, 29.95%, respectively, $p\leq0.05$). None of these changes were observed in the control group of calves. The total number of $CD4^+$ cells on the 18th day of the treatment was conspicuously higher in the treated group ($4.9x10^9$/L, $3.2x10^9$/L, respectively, $p\leq0.05$) than in the untreated animals. The concentrations of PGE_2, PGF_{2a}, and TXB_2 were generally lower in the treated animals; at some points of the measurement the differences were statistically significant. In the case of LTB_4, no tendency towards lower or higher concentrations was observed.

Nowakowska *et al.* (2010) evaluated the *in vivo* influence of ***Uncaria tomentosa*** **bark aqueous and 96% ethanol extract** (DER 1:10) on the metabolic activity of blood granulocytes in mice. Mice were fed for 7 days with both of extracts 200 µg daily, control group was administered vehicle. The metabolic activity of granulocytes was determined by the measurement of their chemiluminescent activity in scintillation counter, after stimulation by Zymosan. Results showed that the water extract highly stimulated the granulocyte chemiluminescence compared to control (25948 Cmp/1000 granulocytes vs. 12506 Cmp/1000 granulocytes, respectively, $p<0.001$) whereas the ethanol extract didn't reach the level of significance. However the ethanol extract had a tendency to decrease the number of leukocytes compared to control (5830/mm^3 vs. 7610/mm^3 respectively, $p<0.1$).

A dried **ethanolic extract (200 g drug, 20 hours, 20^{o}C, DER 1:10, ethanol 30%)** from the bark of *Uncaria tomentosa* was evaluated as a potential immunostimulator. BALP/c mice (n=6 in each group) were seven times immunised intragastrically (i.g.) during 14 days with a formalin-inactivated whole *Sendai* virus (SV) with the dry extract, in two doses (0.56, 5.6 mg). It was found, that the animals inoculated with 5.6 mg extract, induced higher saliva IgA antibodies. Furthermore, the mice immunised e.g. with SV plus 0.56 mg of the extract had significantly higher IgA, IgG and HI (haemagglutination inhibition) antibody responses to SV than did those administered with the SV alone ($p<0.05$). These results suggest that dry extract from bark of *Uncaria tomentosa* is useful as a mucosal adjuvant for mice (Bižanov and Tamošiūnas, 2005).

Domingues *et al.* (2011a) investigated the immunmodulatory potential of a **50% ethanolic extract (no further details are discussed)** of **the bark of** *Uncaria tomentosa* (EUT) (total alkaloid content 29.1 mg/ml) on the progression of immuno-mediated diabetes. C57BL/6 male **mice** were injected with multiple low-doses of streptozotocin (MLDS 40 mg/kg) and treated with EUT at 10–400 mg/kg during 21 days by gavage, *per os*. Control groups received MLDS alone or the respective dilution vehicle. Treating the animals with 50–400 mg/kg of EUT caused a significant reduction in the glycaemic levels, as well as in the incidence of diabetes. Animals treated with EUT at 400 mg/kg presented a higher number of intact islets ($p<0.05$) and a significant inhibition of destructive insulitis. Furthermore, a significant protection against the loss of insulin-secreting presented β-cells was achieved ($p<0.01$). The phenotypic analysis indicated that the groups treated with higher doses (100–400 mg/kg) presented increased numbers of CD4+ and CD8+ T-cell values similar to those observed in healthy animals. These same higher doses also increased the number of CD4+ CD25+ Foxp3+ regulatory T-cells. Moreover, the extract modulated the production of Th1 and Th2, with increased levels of IL-4 and IL-5.

The acute effects of *Uncaria tomentosa* (UT) (**70% ethanolic, spray-dried extract of the ground bark**, 2.57% pentacyclic oxindole alkaloids) in the recovery of neutrophils after chemotherapy-induced neutropenia were assessed, by establishing the correlation with filgrastim (rhG-CSF) treatment to evaluate its possible use in clinical oncology. Ifosfamide-treated mice receiving oral doses of 5 and 15 mg of UT extract and intraperitoneal doses of 3 and 9 µg of filgrastim, respectively, for four days (6 mice/group). The animals were allocated to UT, figrastim and control groups. Bioassays showed that the 5 and 15 mg treatment of UT extract significantly increased the neutrophil count (4- and 13-times higher than control, respectively), and a potency of 85.2% was calculated in relation to filgrastim at the corresponding doses tested. The extract was prepared by ultra-turrax extraction and spray-dried (Farias *et al.* 2011).

Åkesson *et al.* (2003b) found a dose dependent increase in spleen cell numbers in mice (C57BL/6), supplemented with **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) for 24 days, but the proportion of B, T and NK cells, granulocytes, and memory lymphocytes were normal. The absolute number of splenic T cells ($p=0.02$), B cells ($p=0.03$), NK cells ($p=0.009$) and NKT cells ($p=0.02$) had significantly increased, the absolute cell number of granulocytes did not increase. However, there were no detectable changes of the lymphoid architecture of the spleen even after long-term treatment (63 days), but spleen cell number was significantly increased as well. When AE treatment was interrupted, the cellularity returned to normal level within four weeks. AE did not have any significant effect on precursor cells or on the accumulation of recent thymic emigrants in the spleen. Thus, accumulation is most likely due to prolonged cell survival, because adaptive transfer experiments demonstrated that AE treatment significantly prolonged lymphocyte survival in peripheral lymphocyte organs, without increasing their proliferation rate. The experimental groups were fed with AE in their drinking water at approximately daily doses of 125, 250 or 500 mg/kg bodyweight for different periods of time.

Female W/Fu rats (n=8/group) were gavaged daily with **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) of *Uncaria tomentosa* at the doses of 0, 5, 10, 20, 40 and 80 mg/kg for 8 consecutive weeks. PHA stimulated lymphocyte proliferation was significantly increased in splenocytes of rats treated at the doses of 40 and 80 mg/kg. White blood cells (WBC) from the AE treatment groups of 40 and 80 mg/kg for 8 weeks or 160 mg/kg for 4 weeks were significantly elevated compared with controls ($P<0.05$) (Sheng *et al.* 2000a).

The effect of **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) was evaluated in the treatment of doxorubicin (DOX) induced leukopenia in rat model. W/Fu rats (n=11/group) were treated first with DOX 2 mg/kgx3 *i.p.*, then they were daily

gavaged with AE (40 and 80 mg/kg) for 16 days, as a positive control, Neupogen, a granulocyte colony stimulator was used. Both AE and Neupogen treatment groups recovered significantly sooner ($p<0.05$) than DOX group, but in the AE group all fractions of WBC were proportionally increased, while in the Neupogen group mainly neutrophil cells were elevated. DNA repair is possibly involved in these effects (Sheng *et al.* 2000b).

Unknown part

Eberlin *et al.* (2005) demonstrated that *Uncaria tomentosa* extract (UTE) protects mice (BALB/c) from a lethal dose of *Listeria monocytogenes* when administered prophylactically at 50, 100, 150 and 200 mg/kg for 7 days, with survival rates up to 35%. ***Uncaria tomentosa* extract (**UTE, **part not specified**) was provided as a powder (commercial product), dosed in 1% of total alkaloids. These doses also prevented the myelosuppression and the splenomegaly caused by a sublethal infection with *L. monocytogenes*, due to increased numbers of granulocyte-macrophage progenitors (CFU-GM) in the bone marrow ($p<0.001$). Non-infected mice treated with 100 mg/kg UTE also presented higher numbers of CFU-GM in the bone marrow than the controls. Infected and normal mice pre-treated with UTE increased colony-stimulating activity (CSA) in a dose-dependent manner ($p<0.01$ and $p<0.001$ for doses 50 and 100 mg/kg respectively). Increases in the levels of IL-1 ($p<0.05$) and IL-6($p<0.001$) were observed in mice infected with *L. monocytogenes* and treated with 100 mg/kg of UTE. WBC had no significant changes, when infected mice were pre-treated with 100 mg/kg of UTE.

Belteghi and Mânzat (2008) carried out an *in vivo* study, in order to investigate the immunmodulatory virtues of an ***Uncaria tomentosa*-based medicinal product**, by administrating the studied product to an experimental lot (n=7) of domestic rabbits (control lot n=7), and monitoring the dynamics of some immune parameters. The product contained 0.19 g *Uncaria tomentosa*/capsule (no further details are discussed) and the subjects were administered 570 mg of the product daily, via drinking water. The study lasted 32 days, on the 4^{th} and 18^{th} day antigenic inoculations with La Sota strain of *Newcastle* virus were performed to the rabbits of both groups. The haematological results showed that the *Uncaria* product had a stimulating effect upon the total leukocyte count, but this effect was statistically assured only after the second antigenic inoculation of rabbits ($p<0.01$). The inhibo-hemagglutinant titer was, 1.64 fold higher at the final blood sampling, in the experimental lot than in the control lot ($p<0.05$). The phagocitary index showed a substantial, but statistically insignificant (2.16 fold) increase after the first antigenic inoculation only in the experimental lot.

Fractions and mixtures of isolated constituents

Anti-inflammatory properties

In vitro

Bark (not specified)

The anti-inflammatory effects of the non-alkaloid **HPLC fractions from the bark** of *Uncaria tomentosa* (0.1-100 ng/ml) decreased LPS-induced TNF-α and nitrite production in RAW 264.7 cells ($p<0.01$) at a concentration range comparable to the parent botanical. The extraction was performed with MeOH:H_2O:1.2 HCl (50:50:1), and then the **non-alkaloid HPLC fractions** were collected (Sandoval *et al.* 2002).

Immunomodulatory, immunstimulant properties

In vitro

Stem bark

Reis *et al.* (2008) assessed the immunoregulatory effects of **pentacyclic oxindole alkaloid-enriched** (speciophylline, mitraphylline, Uncarine F, pteropodine, isomitraphylline, isopteropodine) or non-alkaloid fractions of **the stem bark** of *Uncaria tomentosa*, which were tested in an *in vitro* DENV (*Dengue* Virus-2) infection model. These fractions were obtained by sonicating (10 minutes) the crude EtOH:H_2O extract (94.2 g) with HCl 0.1 N (1 l), which was then partitioned with EtOAc. Fractions were tested at 0.1-100 µg/ml. No significant alterations were detected with the non-alkaloidal fraction treatment in the measured TNF-α, IL-6, IL-10 and IFN-α levels. However, the alkaloidal fraction inhibited both TNF-α and IFN-α at concentrations of 100 µg/ml and similar cytokine levels resulted with dexamethasone (reference) treatment ($p<0.05$). IL-6, which is strikingly induced after monocyte infection, apparently was not inhibited by *Uncaria tomentosa* or dexamethasone treatments. IL-10 was also hampered significantly by dexamethasone, but there was no significant alteration with the *Uncaria tomentosa* treatment, although a strong tendency for IL-10 inhibition was observed in cultures treated with the alkaloidal fraction: IL-10 levels after DENV infection were 572± 219 pg/ml, lowered to 244±60 pg/ml after treatment and presented ≥18% abrogation in all five PBML donors.

Mixture of compounds/part unknown

The immunmodulatory effect of **two mixtures of tetracyclic** (TOA) **and pentacyclic** (POA) oxindole alkaloids of *Uncaria tomentosa* were investigated in human peripheral mononuclear cells (PBMC) stimulated with the PHA and Con A *in vitro.* Compared to unstimulated cells, PHA and Con A (concanavalin A) increased the production of neopterin and the degradation of tryptophan ($p<0.01$). Mixtures of alkaloids of *Uncaria tomentosa* inhibited both effects in a dose dependant manner, the lowest effective concentrations were 100-175 µg/ml ($p<0.01$). With the highest concentrations complete suppression of mitogen-induced neopterin production and tryptophan degradation was observed, indicating immunmodulating effect of the alkaloids. POA hydrochlorides mixture contained 1% mitraphylline, 49% pteropodine and 50% isopteropodine. TOA hydrochlorides mixture contained 1% isocorynoxeine, 4% corynoxeine, 39% isorhynchophylline (Winkler *et al.* 2004).

Wurm *et al.* (1998) also showed that pentacyclic alkaloids (POA) stimulate endothelial cells *in vitro* to produce a lymphocyte-proliferation-regulating factor. Supernatants of EA.hy926 endothelial cell cultures incubated with 10 µM POA, significantly ($p<0.01$-0.001, depending on dilution of supernatants) increased the proliferation of normal resting or weekly activated human B and T lymphocytes. In contrast, proliferation of normal human lymphoblasts and of both the human lymphoblastoid B cell line Raji CCL86 and the human T cell line Jurkat E6.1 was inhibited significantly ($p<0.01$, 0.001 and 0.005, respectively), while cell viability was not affected. The proliferation of myeloid cell line U-937 was not affected by supernatants of POA stimulated endothelial cell cultures. POA alone did not exert any direct effect on proliferation. Thus the pentacyclic isomers do not affect directly the proliferation but rather induce endothelial cells to release a yet to be identified factor which influences the proliferation of lymphocytes. For generation of supernatants, EA.hy926 cells were incubated with 1 µM POA and TOA. Supernatants of untreated cells served as control. Tetracyclic oxindole alkaloids dose-dependently reduced the activity of pentacyclic oxindole alkaloids on human endothelial cells**.** POA-preparation contained: 4% speciophylline, 6% Uncarine F, 2% mitraphylline, 3% isomitraphylline, 28% pteropodine and 57% isopteropodine. The TOA preparation contained: 67% rhynchophylline and 33% isorhynchophylline. In the studies of antagonistic effects two separate POA preparations were used: pteropodine group (4% speciophylline, 6% Uncarine F, 30% pteropodine,

60% isopteropodine) and the mitraphylline group (33% mitraphylline, 67% isomitraphylline) (Wurm *et al.* 1998; Keplinger *et al.* 1999). Based on this study of Wurm *et al.* (1998), it has been stated, that the mixture of the two chemotypes of cat's claw are unsuitable for therapeutic use, unless certified to contain less than 0.02% tetracyclic oxindole alkaloids (Laus and Keplinger, 1997; Barnes *et al.* 2002).On the other hand, one tetracyclic alkaloid of *Uncaria tomentosa*, isorhynchophylline, was able to induce phagocytosis, while rhychophylline did not affect it (Keplinger *et al.* 1990). Thus isorhynchophylline may act as an immunostimulant, affecting other pathways than via endothelial cells. Hence, caution is advised, before drawing consequences based on one study, especially, concerning the immune system that has plenty probable targets. Moreover, most of the studies of *Uncaria tomentosa* were performed with extracts containing both types of alkaloids (Keplinger *et al.* 1990; Wagner *et al.* 1985).

A standardised extract (3% total alkaloids, commercial product, no further details) of ***Uncaria tomentosa*** **(part not specified**) was evaluated for ability to activate macrophage and natural killer cells, *in vitro* by Groom *et al.* (2007). Macrophage phagocytosis was stimulated up to 4.7-fold, ($P<0.01$) at concentrations 0.128, 0.385, and 1.28 mg/ml. NK cell synthesis of interferon-γ, macrophage synthesis of IL-12 or NK cell synthesis of granzyme B were not stimulated.

In vitro+in vivo

Domingues *et al.* (2011b) investigated the effects of a **pentacyclic oxindole alkaloid** (POA) extract derived from a 50% ethanolic extract of the ***Uncaria tomentosa*** **bark** on lymphocyte phenotype, Th1/Th2 cytokine production, cellular proliferation and cytotoxicity. POA extract was prepared by treatment of the crude 50% ethanolic extract with 0.1 N HCl and then partitioned with EtOAc. The resulting aqueous fraction was treated with NH_4OH until a pH of 9–10 was reached, filtered and evaporated. For the *in vivo* immunotoxicity testing, BALB/c male mice (10/group) were treated once a day with 125, 500 or 1250 mg/kg of POA extract for 28 days. The extract increased the cellularity of splenic white pulp and the thymic medulla and increased the number of T helper lymphocytes and B-lymphocytes. The animals treated with 1250 mg/kg bodyweight of POA displayed a significant increase in the relative number of lymphocytes (91.4%, $p<0.05$), which was associated with a decrease in granulocytes (5.5%, $p<0.01$), compared to control (82% and 16.1% respectively). Also, a large stimulatory effect on lymphocyte viability was observed. However, *in vitro* mitogen induced (Con A) T lymphocyte proliferation was significantly inhibited at higher concentrations of the POA extract ($p<0.05$), concentrations tested 10, 50, 100 and 500 µg/ml. Furthermore, an immunological polarisation toward a Th2 cytokine profile was observed. POA extract increased the mitogen-induced production of IL-4 ($p<0.01$) and IL-5 ($p<0.001$) and caused a strong inhibition of IFN-γ ($p<0.001$) at the highest tested concentration (500 µg/ml). In addition both concentrations of 100 and 500µg/ml inhibited the production of IL-2. Despite the absence of changes in the TNF-α level at 500µg/ml, the lowest tested concentration (100 µg/ml) led to a significant increase ($p<0.01$).

Isolated constituents

Anti-inflammatory properties

In vitro

From the bark of *Uncaria tomentosa* (200 g extracted with petroleum ether) Wirth and Wagner (1997) isolated Cinchonain 1b which inhibited 5-lipoxygenase (>100% at 42.5 µM/ml).

In vivo

Aquino *et al.* (1991) reported, that the bioassay-guided fractionation of the petroleum ether extracts of the root bark of *Uncaria tomentosa*, using the carrageenan-induced oedema in rat (male Wistar Nossan) paw, has led to the isolation of a **new quinovic acid glycoside**, 3-β-*O*-(β-D-quinovopyranosil)-(27→I)-β-D-glucopyranosyl ester, as one of the active principles. The oral pre-treatment with the compound caused 33% inhibition at 3 hours of the inflammatory response ($p<0.05$). Subjects (n=5/group) received either indomethacin (5 mg/kg), vehicle or the pure compound at 20 mg/kg. One hour after drug administration 1% carrageenan was injected and the paw volumes were measured hourly for 5 hours.

Mitraphylline a major pentacyclic oxindole alkaloid, present in the bark chloroformic extract of *Uncaria tomentosa* was tested *in vivo* against a large range of cytokines that play a crucial role in inflammation. Mice received mitraphylline once a day for 3 days at 30 mg/kg/day by oral route. Then they were subjected to LPS endotoxin (15 mg/kg) and the LPS-induced production of 16 different cytokines was determined by Elisa multiplex. Control group received dexamethasone orally at 2 mg/kg/day. The control group received 0.9% saline solution, while dexamethasone (2 mg/kg/day, oral route) was used as reference. Two hours after the last dose, mice were injected intraperitoneally with saline-diluted LPS, then two hours later sacrificed. Mitraphylline inhibited around 50% of the release of interleukins 1α, 1ß, 17, and TNF-α. This activity was similar to dexamethasone. It also reduced almost 40% of the production of interleukin 4, while the corticoid did not (Rojas-Duran *et al.* 2012).

Immunmodulatory, immunostimulant properties

In vitro+In vivo

Wagner *et al.* (1985) reported that **six oxindole alkaloids** from *Uncaria tomentosa* (pteropodine, isopteropodine, rhynchophylline, isorhynchophylline, mitraphylline, isomitraphylline) showed a pronounced enhancement effect on phagocytosis. Isopteropodine (10^{-3}-10^{-5}% concentration) had the greatest effect with an elevation of 23.4-55% of the phagocytosis index, in the granulocytic test. Isopteropodine, isorhynchophylline and isomitraphylline were also active with a lesser extent (10.8%-27%), but mitraphylline and rhynchophylline were inactive. However, *in vivo* carbon clearance test, where an aqueous extract in the form of a water-soluble hydrochloric of the mixed alkaloids of *Uncaria tomentosa* administered to rats (10 mg/kg, i.p.), have shown that they are not active without the presence of the catechin tannin fraction of the root bark, even though these catechins were shown to be immunologically inactive. In another study speciophylline stimulated granulocyte phagocytosis *in vitro* by 25% to 35%. Among the tetracyclic oxindoles, dihydrocorynantheine, hirsutine and hirsuteine also stimulated granulocyte phagocytosis by 25% to 35% (McKenna *et al.* 2002).

Åkesson *et al.* (2005) reported, that mice exposed to **quinic acid** (QA), a component of **a commercially available filtered aqueous extract** (AE), had significantly increased number of spleen cells, thus recapitulating the *in vivo* biological effect of AE (which was repeated here). Mice were treated with 1, 2 and 4 mg/ml QA for 21 days (n=9-24). Ammonia treated quinic acid (QAA) but not QA, inhibited proliferation of mitogen-stimulated 70Z/3 mouse pre-B lymphocytes. Spleen cells were activated with Con A or LPS in the presence of concentrations of 1 mg/ml AE, QA or 1 and 2 mg/ml QAA. Both QA and AE inhibited NF-κB activity in exposed Jurkat T cells at similar concentrations (0.625-2.5 mg/ml).

Table 4: Overview of the main non-clinical data/conclusions

	Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Herbal preparations					
Anti-inflammatory					
Bark	Aqueous extract of dried bark of *U. tomentosa* (20 g/L)	100 µg/ml In the case of iNOS gene expression 50-200 µg/ml	*In vitro* HT29 and RAW 264.7 cells	Sandoval-Chachón 1998	Inhibited peroxynitrite-induced apoptosis, LPS induced iNOS gene expression, nitrite formation and cell death and the activation of NF-κB in in the presence of LPS ($p<0.05$)
Bark	Aqueous extract of micropulverised bark of *U. tomentosa* (20 g/L) with or without concentration by freeze-drying	Freeze-dried: 0.001-3 µg/ml Without concentration 0.01-100 µg/ml	*In vitro* RAW 264.7 cells	Sandoval 2000	Suppressed LPS induced TNF-α production by 65-85% ($p<0.01$) freeze-dried EC_{50}=1.2 ng/ml, without freeze-drying EC_{50}=28 ng/ml
Bark	Aqueous extract of the bark of *U. tomentosa* (AT) and *U. guianensis* (AG)(50 g/L)	Freeze-dried extracts diluted to 20 mg/ml	*In vitro* RAW 264.7 cells	Sandoval 2002	The IC_{50} of TNF-α production was higher for AT (14.1 ng/ml) vs. AG (9.5 ng/ml), $p<0.01$
Bark	Two *U. tomentosa* bark extracts: 80% ethanolic spray-dried extract (DER 8:1) and aqueous freeze-dried	5-500 µg/ml	*In vitro* NF-kB EMSA (Jurkat T cells) COX1 and 2 assays.	Aguliar 2002	NF-kB activation was nearly completely reduced with 500 µg/ml of the hydroalcoholic extract but aqueous extract only slightly reduced it. COX-1 and 2 inhibition: 7.8% and 21.7% (hydroalcoholic) 32.7% and 12.2% (aqueous freeze-dried)
Stem bark	Purified freeze-dried extracts of stem bark	1–1000 ng/ml or 50 ng/ml	*In vitro* HT29 and RAW 264.7 cells	Piscoya 2011	Significant and dose-dependent reduction in TNF-α levels after stimulation, equivalent between *Uncaria tomentosa and guaianensis* IC_{50} values (10.2, 10.9 ng/ml, respectively) and with a maximum inhibition of 79% and 73%, respectively. The extracts had no effect on unstimulated PGE2 production but did significantly reduce PGE2 production stimulated by LPS.
Bark	Aqueous extract of dried bark of *U. tomentosa* (20 g/L)	5 mg/l, orally in drinking water	*In vivo* Sprague-Dawley rats, with indomethacin induced intestinal inflammation	Sandoval-Chachón 1998	The extract attenuated indomethacin enteritis: it reduced the myeloperoxidase activity, morphometric damage and metallothionein expression ($p<0.05$)
Bark	Aqueous extract of the bark of *U. tomentosa* (UT) (20 g\L) DER 1:14	50% and 100% UT extract, per os Drinking water ad libitum Pre-treatment	*In vivo* Mice (n=32/group) ozone inhalation caused acute pneumonitis	Cisneros 2005	Lung tissue protective effect of pre-treatment: Significantly ($p<0.05$) lower levels of protein in BALF, lower degree of epithelial necrosis, higher number of intact epithelial cell nuclei in bronchial wall, and decreased number of PMNs in the bronchiolar lumen

	Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Bark	Two *U. tomentosa* bark extracts: 80% ethanolic spray-dried extract (DER 8:1) and aqueous freeze-dried *U. tomentosa* extract	500, 200, 100 and 50 mg/kg Gastric probe during 8 days Pre-treatment	*In vivo* Carrageenan-induced paw edema model in BALB/c mice	Aguliar 2002	The pre-treatment with hydroalcoholic extract (50 mg/kg) produced an anti-inflammatory effect similar to 7 mg/kg of the non-steroidal drug indomethacin, while the aqueous freeze-dried extract exhibited the same effect at 200 mg/kg.
Bark	Micropulverised bark of *U. tomentosa*	5 mg/l, orally in drinking water Pre-treatment	*In vivo* Sprague-Dawley rats, with indomethacin induced gastritis	Sandoval 2002	Protective effect ($p<0.01$), the degree of gastric mucosal injury was markedly attenuated TNF-α mRNA expression and apoptosis was prevented
Root bark	Fractions from the petroleum ether extract of the root bark of *U. tomentosa*	*Per os* $CHCl_3$/MeOH (9:1) (50 mg/kg) Water (84 mg/kg) MeOH and $CHCl_3$ fractions (doses equivalent to 2 g of dry bark/kg) Pre-treatment	*In vivo* Carragenan-induced edema in normal and in normal and indomethacin treated Wistar rats (n=5/group)	Aquino 1991	Pre-treatment with the fractions ($CHCl_3$/MeOH (9:1) and water) caused 69.2 and 41.2% inhibition at 3 hours of the inflammatory, respectively.
Root bark	Decoction of *U. tomentosa* root bark	Pre-treatment with 3 ml of the added to drinking water	*In vivo* Rats	McKenna 2002	The size and number of large gastric ulcers were significantly inhibited compared to controls
Root	Aqueous (70°C) extract of the root of *U. tomentosa* (10% w/v), then freeze-dried	250 or 500 mg/kg extract i.p. Pre-treatment	*In vivo* Sprague-Dawley rats (n=6/group) Edema induced by *Bothrops asper* snake venom	Badilla 2006	The doses of 250 mg/kg and 500 mg/kg showed an anti-inflammatory effect at all times ($p<0.05$), except for the dose of 250 mg/kg at one hour
Unknown	70% ethanolic extract of *U. tomentosa*	0.125, 0.5, 2 g/kg *Per os* Post-treatment	*In vivo* Carragenan-induced edema in normal and in prednisolone treated mice (n=5)	Abe 2002	The post-treatment significantly ($p<0.05$) lowered the thickness of the edema administered together *with Harpagophytum procumbens*, but not alone
Immunmodulatory					
Bark	*U. tomentosa* (70% ethanolic extract of the bark, 2.57% pentacyclic oxindole alkaloids)	100 and 200 µg/ml	*In vitro* Colony-forming cell assays performed with hHSPCs	Farias 2011	Increase in CFU-GM size and mixed colonies (CFU-GEMM) size at the final concentrations of 100 and 200 µg extract/ml
Bark	95% ethanolic extract of the bark of *U. tomentosa* (exhaustive percolation)	1 or 10 µl (concentration not given)	*In vitro* LPS-treated THP-1 monocyte cells	Allen-Hall 2007	Augmented the expression of IL-1β by 20-fold, but did not affect the expression of TNF-α, ($p<0.05$)

	Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Bark	95% ethanolic extract of the bark of *U. tomentosa* (exhaustive percolation)	10-360 µg/ml	*In vitro* LPS-treated THP-1 monocyte cells	Allen-Hall 2010	The treatment: Inhibited the secretion of TNF-α in a dose-dependent manner (33-95%) Enhanced the expression of IL-1β at 40 and 160 µg/ml but blocked at 320 µg/ml. Inhibited the activation of all AP-1 transcription factor subunits but only inhibited some of the NF-κB subunits ($p<0.05$)
Stem bark	Two stem bark aqueous extracts of different collections of *U. tomentosa*: AAT and MUT -6.98 and 5.57 mg/g total oxindole alkaloid content respectively	0.025-0.5 mg/ml	*In vitro* Alveolar rat macrophages with or without LPS stimulation	Lemaire 1999	MUT greatly stimulated IL-1 and IL-6 (10X and 7.5X at max. effect) production in a dose dependent manner and enhanced IL-1 (5.2X) and IL-6 (<2X) in LPS stimulated macrophages. AAT had comparable trends in results (not specified)
Stem bark	50% ethanolic extract of the stem bark of *U. tomentosa*	0.1-100 µg/ml	*In vitro* DENV (*Dengue* Virus-2) infection model.	Reis 2008	No significant alterations were detected with the treatment of hydro-ethanolic extract in the measured TNF-α, IL-6, IL-10 and IFN-α levels.
Stem bark	70% ethanolic extract of the stem bark of *U. tomentosa* (48 hours. 25°C)	250-3.9 µg/ml in complement cascade, 5 mg/ml in ADP-induced platelet aggregation assay	*In* vitro Complement cascade inhibition and ADP-induced platelet aggregation inhibition assays.	Deharo 2004	Inhibition of the classical-CPW and alternative-APW complement pathways, IC_{50}: 124 and 151 µg/ml, respectively. ADP-induced platelet aggregation-no inhibition
Stem bark	Commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE)	Several concentrations in different assays: 0.125-2 mg/ml	*In vitro* Mouse CTLL-2 cells for IL-2 assay, mouse spleen cells, IL-2 ELISA, LPS stimulated 70Z/3 and Jurkat cells for NF-κB	Åkesson 2003a	The extract inhibited the proliferation of normal mouse T and B-lymphocytes and the inhibition is not caused by toxicity or by induction of apoptosis. It did not interfere with IL-2 production nor IL-2 receptor signalling, but reduced NF-κB activity with almost 50%.
Stem root bark	Extract of the stem or root bark of *U. tomentosa*		*In vitro* Granulocytic test	McKenna 2002	Increase in phagocytic activity shown by the root bark was found to be 30% to 40% Increase in phagocytic activity shown by the stalk bark was found to be 10% to 20%
Unknown	Aqueous (roomtemp.) extract of *U. tomentosa* (commercial product) 10 ml/g	44 µg/ml	*In vitro* FACS-based IL-2Rα and CFSE analysis of human and bovine PBMC	Holderness 2007	IL-2Rα activation, proliferation and expression on γδ T cells were induced. The activity was specific to γδ T cells and was not limited to one γδ T cell subset. The activity was due to the condensed tannin fractions of the drug.
Unknown	HCl and 96% ethanolic *U. tomentosa* extracts (commercial products)	Concentration range of 500-4000 µg/ml	*In vitro* Human peripheral mononuclear cells (PBMC) stimulated with the PHA and Con A	Winkler 2004	The extracts inhibited the production of neopterin and the degradation of tryptophan in a dose dependant manner; the lowest effective concentrations were 500-1000 µg/ml ($p<0.05$). At the highest concentrations, complete suppression was observed, indicating immunomodulation effect of the extracts.

	Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Bark	Decoction of the bark of *U. tomentosa*	Group I (n=10, 3600 mg/calf/day) and Group II (n=10, placebo) *Per os* 3600 mg in 200 ml decoction form, for 17 day	*In vivo* Calves (n=10/group)	Bedranek 2002	In the treatment group the peripheral blood neutrophil and MID-cell number and the rectal temperature were significantly lower, the peripheral lymphocyte subsets increased significantly in the total number and % of CD2 + and CD4+ cells and the synthesis and release of pro-inflammatory arachidonate metabolites (eicosanoids) were also inhibited.
Bark	Water and 96% ethanol extract of *U. tomentosa* bark (DER 1:10)	200 µg daily for 7 days *Per os*	*In vivo* BALB/c mice	Nowakowska 2010	Metabolic activity of granulocytes was stimulated by the water extract compared to control ($p<0.001$). Ethanol extract didn't reach the level of significance.
Bark	30% ethanolic extract of *U. tomentosa* bark (Drug solvent ratio 1:10)	0.56, 5.6 mg of the dry extract seven times Intragasrically	*In vivo* BALB/c mice (n=6/group) immunised with a formalin-inactivated whole Sendai virus (SV) with two doses of extract.	Bižanov 2005	Animals inoculated with 5.6 mg of the dry extract induced higher saliva IgA antibodies. Mice immunised e.g. with SV plus 0.56 mg of the extract had significantly higher IgA, IgG and HI (haemagglutination inhibition) antibody responses to SV than did those administered with the SV alone ($p<0.05$)
Bark	50% ethanolic extract of the bark of *U. tomentosa* (EUT) Total alkaloid content 29.1 mg/ml	10–400 mg/kg during 21 days By gavage, *per os*	*In vivo* immune-mediated diabetes in C57BL/6 male mice	Domingues 2011a	Insulinitis protective effect: 50–400 mg/kg of EUT caused a significant reduction in the glycemic levels and incidence of diabetes, protection against the loss of β-cells ($p<0.01$) and higher number of intact islets ($p<0.05$) Extract modulated the production of Th1 and Th2, with increased levels of IL-4 and IL-5 ($p<0.05$).
Bark	*U. tomentosa* (70% ethanolic extract of the bark, 2.57% pentacyclic oxindole alkaloids)	5 and 15 mg of UT extract *per os* and intraperitoneal doses of 3 and 9 µg of filgrastim, respectively, for 4 days.	*In vivo* Ifosfamide-treated mice (n=6/group)	Farias 2011	Treatment significantly increased the neutrophil count (4 and 13X higher than control, respectively), and a potency of 85.2% was calculated in relation to filgrastim at the corresponding doses tested.
Bark	Commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE)	Approximately daily doses of 125, 250 or 500 mg/kg bodyweight for different periods of time (63, 24 days), in drinking water	*In vivo* C57BL/6 mice (7/group)	Åkesson 2003b	A dose dependent increase in spleen cell numbers, but the proportion of B, T and NK cells, granulocytes, and memory lymphocytes were normal. Accumulation is most likely due to prolonged cell survival
Bark	Commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE)	0, 5, 10, 20, 40 and 80 mg/kg for 8 or 4 consecutive weeks *Per os*	*In vivo* Female W/Fu rats (n=8/group)	Sheng 2000a	Lymphocyte proliferation was significantly increased in splenocytes of rats treated at the doses of 40 and 80 mg/kg. White blood cells (WBC) were significantly elevated compared with controls ($P<0.05$)
Bark	Commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE)	40 and 80 mg/kg for 16 consecutive days *Per os*	*In vivo* Female W/Fu rats (n=11/group)	Sheng 2000b	Both AE and Neupogen (positive control) treatment groups recovered significantly sooner ($p<0.05$) than DOX group, but in AE all fractions of WBC were proportionally increased, while in Neupogen group mainly neutrophil cells elevated.

	Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Unknown	*U. tomentosa* extract (UTE) (not specified) was provided as a powder, dosed in 1% of total alkaloids.	50, 100, 150 and 200 mg/kg for 7 days *Per os*	*In vivo* BALB/c mice inoculated i.p. with lethal and sublethal dose of Listeria *monocytogenes*	Eberlin 2005	Increased survival rates up to 35% Increased numbers of (CFU-GM) in the bone marrow ($p<0.001$). Increased colony-stimulating activity (CSA) in dose-dependent manner ($p<0.01$ and $p<0.001$ for doses 50 and 100 mg/kg respectively). Higher numbers of CFU-GM in non-infected mice, 100 mg/kg UTE. WBC had no significant changes Increased levels of IL-1 ($p<0.05$) and IL-6 ($p<0.001$) at 100 mg/kg
Unknown	Uncaria product (0.19 g *U. tomentosa*/capsule)	570 mg/product/day from capsules for 32 days in drinking water	*In vivo* Domestic rabbits, 2 antigenic inoculations with La Sota strain of *Newcastle* virus (n=7/group)	Belteghi 2008	*Uncaria* product had a stimulating effect upon the total leukocyte count ($p<0.01$), inhibo-hemagglutinant antibody titer was 1.64 fold higher than in the control lot ($p<0.05$). Phagocitary index had a substantial, but statistically not significant (2.16 fold) increases after the first inoculation
Fractions and mixtures of isolated constituents					
Anti-inflammatory					
Bark	MeOH:H_2O:1.2 HCl (50:50:1) extract of the bark of UT, then non-alkaloid HPLC fractions were collected	Non-alkaloid HPLC fractions from UT (0.1-100 ng/ml)	*In vitro* RAW 264.7 cells	Sandoval 2002	Decreased LPS-induced TNF-α and nitrite production ($p<0.01$)
Immunmodulatory					
Stem bark	Pentacyclic oxindole alkaloid-enriched (speciophylline, mitraphylline, Uncarine F, pteropodine, isomitraphylline, isopteropodine) or non-alkaloid fractions	0.1-100 µg/ml	*In vitro* DENV (*Dengue* Virus-2) infection model.	Reis 2008	No significant alterations were detected with the non-alkaloidal fraction treatment in the measured TNF-α, IL-6, IL-10 and IFN-α levels. Alkaloidal fraction inhibited both TNF-α and IFN-α at concentrations of 100 µg/ml and similar cytokine levels resulted with the dexamethasone treatment. Il-6 was not inhibited neither DEX nor the alkaloidal fraction.
Unknown	Two mixtures of tetracyclic (TOA) and pentacyclic (POA) oxindole alkaloids of *U. tomentosa*	Concentration range of 50-500 µg/ml	*In vitro* Human peripheral mononuclear cells (PBMC) stimulated with the PHA and Con A	Winkler 2004	The production of neopterin and the degradation of tryptophan were inhibited in a dose dependant manner, the lowest effective concentrations of 100-175 µg/ml ($p<0.01$). At the highest concentrations complete suppression was observed, indicating immunmodulating effect of the alkaloids.

	Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Unknown	Pentacyclic oxindole alkaloids (POA) Tetracyclic oxindole alkaloids (TOA)	Generation of supernatant: 1µM POA Proliferation assays: supernatants were tested at dilutions from 1:2-1:32.	*In vitro* EA.hy926 endothelial cells, proliferation assays (B and T lymphocytes, T and B blasts, Raji CCL86 and JurkatE6.1)	Wurm 1998	POA induced EA.hy926 endothelial cells to release unknown factor(s) into supernatant; which significantly enhanced proliferation of normal human B and T lymphocytes; and inhibited the proliferation of normal human lymphoblasts, lymphoblastoid B cell line Raji and T cell line Jurkat ($p<0.01$, 0.001 and 0.005, respectively) TOA dose-dependently reduced the activity of POA on human endothelial cells
Unknown	Standardised extract (3% total alkaloids)	0.128, 0.385, and 1.28 mg/ml	*In vitro* II-12, IFN- γ ELISA, Vibrant phagocytosis assay, ELISPOT granzyme B assay	Groom 2007	Macrophage phagocytosis was stimulated up to 4.7-fold, ($P<0.01$) at all concentrations, NK cell synthesis of interferon-γ, macrophage synthesis of interleukin-12 or NK cell synthesis of granzyme B were not stimulated.
Bark	Pentacyclic oxindole alkaloid (POA) extract from 50% ethanolic extract of the *U. tomentosa* bark	10, 50, 100 and 500 µg/ml for proliferation, 100 and 500 µg/ml for cytokine production	*In vitro* Mitogen induced (Con A) T lymphocyte proliferation, T1, 2 cytokine production (IL5, 2, 4, TNF-α, IFN-γ)	Domingues 2011b	Mitogen induced T lymphocyte proliferation was significantly inhibited at higher concentrations of the extract ($p<0.05$). Increased mitogen-induced production of IL-4 ($p<0.01$) and IL-5 ($p<0.001$), inhibition of IFN-γ ($p<0.001$) at the highest tested concentration (500 µg/ml), inhibition of the production of IL-2, No changes in the TNF-α level at 500µg/ml, but significant increase at 100µg/ml ($p<0.01$)
Bark	Pentacyclic oxindole alkaloid (POA) extract from 50% ethanolic extract of the *U. tomentosa* bark	125, 500 or 1250 mg/kg bodyweight of *U. tomentosa* extract for 28 days *Per os*	*In vivo* BALB/c male mice (10/group)	Domingues 2011b	Increased cellularity of splenic white pulp and the thymic medulla Increased relative number of lymphocytes (91.4%, $p<0.05$), associated with a decrease in granulocytes (5.5%, $p<0.01$), compared to control (82% and 16.1% respectively). Stimulatory effect on lymphocyte viability was observed.
Isolated constituents					
Anti-inflammatory					
	Cinchonain 1b	42.5 µM/ml	*In vitro*	Wirth 1997	Inhibited 5-lipoxygenase (>100%)
	Quinovic acid glycoside, 3-β-*O*-(β-D-quinovopyranosil)-(27→I)-β-D-glucopyranosyl from the petroleum ether extract of the root bark of *U. tomentosa*	*Per os* 20 mg/kg	*In vivo* carragenan-induced edema in normal and in normal and indomethacin treated Wistar rats (n=5/group)	Aquino 1991	33% inhibition at 3 hours of the inflammatory response.

	Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
	Mitraphylline	3 days pre-treatment 30 mg/kg/day *Per os*	*In vivo* LPS-induced production of 16 different cytokines was determined in BALB/c mice by Elisa multiplex	Rojas-Duran 2012	Inhibition of approximately 50% of the release of interleukins 1α, 1ß, 17, and TNF-α. This activity was similar to dexamethasone. It also reduced almost 40% of the production of interleukin 4, while the corticoid did not
Immunology					
	Pteropodine, isopteropodine, rhynchophylline, isorhynchophylline, mitraphylline, isomitraphylline	10^{-3}-10^{-5}% concentration	*In vitro* Granulocytic test (phagocytosis test)	Wagner 1985	Isopteropodine had the greatest effect with an elevation of 23.4-55% of the phagocytosis index. Isopteropodine, isorhynchophylline and isomitraphylline were also active with a lesser extent (10.8%-27%), but mitraphylline and rhynchophylline were inactive.
	Speciophylline, dihydrocorynantheine, hirsutine and hirsuteine		*In vitro* Granulocytic test (phagocytosis test)	McKenna 2002	Speciophylline stimulated granulocyte phagocytosis by 25% to 35%. Dihydrocorynantheine, hirsutine and hirsuteine also stimulated granulocyte phagocytosis by 25% to 35%.
	Quinic acid (QA) Ammonia treated quinic acid (QAA)	0.625-2.5 mg/ml	*In vitro* Jurkat cells 70Z/3 mouse pre-B lymphocytes	Åkesson 2005	QAA, but not QA, inhibited proliferation of mitogen-stimulated mouse lymphocytes., QAA or QA. Both QA and AE inhibited NF-κB activity.
	Pteropodine, isopteropodine, rhynchophylline, isorhynchophylline, mitraphylline, isomitraphylline	10 mg/kg, i.p.	*In vivo* Carbon clearance test Rats	Wagner 1985	They are not active without the presence of the catechin tannin fraction of the root bark, even though these catechins were shown to be immunologically inactive.
	Quinic acid (QA)	1, 2 and 4 mg/ml in drinking water for 21 days	*In vivo* C57BL/6 mice (n=9-24)	Åkesson 2005	Increased number of spleen cells

3.1.2. Secondary pharmacodynamics

Antiproliferative properties and mechanisms

In vitro

The alkaloid class of *Uncaria tomentosa* was the most commonly, mainly *in vitro* tested group across studies and provided limited experimental evidence demonstrating anti-proliferative effects, maybe through pro-apoptotic pathways (Gurrola-Diaz *et al.* 2011; De Martino *et al.* 2006). The antiproliferative effects of different extracts (H_2O or 25, 50, 96% ethanol or alkaloid enriched) of the bark were tested on several cell-lines (HT-29, SW 707, KB, MCF-7, A-549, OAW-42, HL-60, LLC, B16, Neuro-2a, T24, RT4, CCL-86, CCL-240, TIB-152, K-562, SAOS-2) (Mazzio and Soliman, 2009; Pilarski *et al.* 2007 and 2010; Kaiser *et al.* 2013; Åkesson *et al.* 2003a; Sheng *et al.* 1998; De Martino *et al.* 2006). The inhibitory activities were weak to moderate; the IC_{50} values of an alkaloid rich fraction and an ethanolic extract of the bark on the KB (human cervical carcinoma) and LLC (LL/2, mouse Lewis lung carcinoma) cell lines were the lowest, 23 and 25 µg/ml, respectively (Pilarski *et al.* 2010). The alkaloid content in preparations increased with the ethanol concentrations reaching the highest value at 96% ethanol. It was also observed that the inhibition showed a high correlation between the total oxindole alkaloid content and the antiproliferative activity of the preparations. Oxindole alkaloids from *Uncaria tomentosa,* exhibited weak to moderate activity with IC_{50} values ranging between 11.8-51 µM, or displayed no activity at all. Mitraphylline had the lowest IC_{50} values among all the tests performed with different alkaloids on different cells (Gimenez *et al.* 2010; Stuppner *et al.* 1993; Prado *et al.* 2007; Bacher *et al.* 2005; Åkesson *et al.* 2005; Lee *et al.* 1999; Muhammad *et al.* 2001).

In vivo

The activity of *Uncaria tomentosa* preparations with different **quantitative and qualitative oxindole alkaloid compositions** were studied by Pilarski *et al.* (2010) on cancer cells using ***in vitro*** and ***in vivo*** models. The **bark of *Uncaria tomentosa*** was extracted with **water or ethanol (50 or 96%)** on different temperatures (37°C and boiling) and an alkaloid rich fraction (B/SRT) was prepared as well. Animal studies on mice (21 days, 5 and 0.5 mg daily doses/sample, 6 mice/group) with LLC showed significant inhibition of tumour growth by the aqueous extract (37°C), which exhibited the lowest toxicity *in vitro*. In contrast the activity of B/96E37 and B/SRT preparations (most effective *in vitro*) did not reveal any significant anti-tumour activity. The two control groups were treated with physiological salt solution and DMSO 10%.

The proliferative effect of a **70% ethanolic bark** extract of *Uncaria tomentosa* (BHE) (1 hour extraction at 20°C, concentrated, dried, atomised, total alkaloid content was of 5.03%) was demonstrated in the Walker-256 cancer model by Dreifuss *et al.* (2010). Walker-256 tumour cells were subcutaneously inoculated in the pelvic limb of male Wistar rats. Daily gavage with BHE (10, 50 or 100 mg/kg, Groups BHE, n=9) or saline solution (Control, Group C, n=9) was subsequently initiated, until 14 days afterwards. For some parameters, a group of healthy rats (Baseline, Group B, n=5) was added. Compared to control group BHE reduced the tumour volume by 46%, 58% and 64% at doses of 10, 50 or 100 mg/kg, respectively. In addition, treatment with BHE reduced the activity of AST, but did not reverse the increase of LDH and GGT plasma levels, although all doses effectively reduced urea plasma levels. Treatment also resulted in increased CAT activity in liver, while decreasing it in tumour tissue. SOD activity was reduced in liver as well as in tumour, compared to Group C. The antineoplastic activity may result, partially at least, from the ability of BHE to regulate redox and metabolism homeostasis.

The antineoplastic properties **of *Uncaria tomentosa* 70% ethanolic (BHE) bark extract** (1 hour extraction, concentrated, total alkaloid content was of 5.03%) and its two fractions choroformic ($CHCl_3$) and n-butanolic (BuOH) were demonstrated and compared by Walker-256 cancer model. Subsequently to the inoculation, gavage with BHE extract (50 mg/kg) or its fractions (extrapolated doses for both fractions) or vehicle (Control) was performed during 14 days. Baseline values, corresponding to individuals without tumour or treatment with BHE, were also included. The number of rats (n) in each of these groups was 4–6. Both the BHE and its BuOH fraction (rich in antioxidant substances) successfully reduced tumour weight and volume (tumour mass suppression of 52% and 49%, respectively, p=0.01) and modulated anti-oxidant systems. Inversely, the $CHCl_3$ fraction (rich in pentacyclic oxindole alkaloids) was ineffective. The tumour volume suppression rates of the BHE and BuOH fraction groups as compared to the control group were 52% and 58% respectively, while the $CHCl_3$ fraction had similar values as the control group. Both the BHE and its BuOH fraction increased the survival time of the tumour-bearing animals. The BHE treated group survived the entire observation period (30 days), but no individual belonging to the control and $CHCl_3$ groups remained alive at the end of the trial (Dreifuss *et al.* 2013).

DNA repair

In vitro

Bark (not specified)

Extracts (petroleum ether, chloroform, chloroform:methanol 9:1, methanol, water) and chromatographic fractions (Sephadex LH-20 of chloroform/methanol extract) of *Uncaria tomentosa* **bark** showed no mutagenic effect in different strains of *Salmonella typhimurium* with and without metabolic activation. However, the plant extracts and fractions showed a protective antimutagenic effect (AMES test) *in vitro* against photomutagenesis in *S. typhimurium* TA 102. The chloroformic and methanolic extracts were the less (27%) and the most (59%) active extracts (Rizsi *et al.* 1993).

Skin cultures co-incubated with **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE, 5 mg/ml) reduced skin cell death from UV exposure compared to no treatment (p<0.001), and this protection was accounted for by a concomitant increase in DNA repair measured by TT dimmers DNA lesions. The percent reduction in TT-dimers was found to be statistically significant in the AE group compared with the untreated controls (73% vs. 11%, respectively, $p \leq 0.001$) (Mammone *et al.* 2006).

Stem bark

Antimutagenic activity of *Uncaria tomentosa* maceration extract (UT mac), *Uncaria tomentosa* plus *Uncaria guianensis* maceration extract (UT+UG mac), *Uncaria tomentosa* reflux extract plus *Uncaria guianensis* reflux extract (UT+UG ref), oxindole alkaloid purified fraction (OAPF, 62.73% POA) and quinovic acid glycosides purified fraction (QAPF 217 µg/mg) were evaluated by Caon *et al.* (2014). Maceration and reflux extraction of the **stem bark** samples were performed with 40% ethanolic solution (DER 1:10). The protective effect of samples at 1000 µg/ml (non-cytotoxic concentration) on UV-induced DNA damage was assessed using the Comet assay with minor modifications. The simultaneous treatment provided greater protective effect on UV-induced DNA damage (reaching protection levels of 75%) than the pre-, or post-treatment, and this effect clearly showed to be dependent on sample concentration (250–1000 µg/ml). Both purified fractions (QAPF and OAPF) showed lower protective effect on UV-induced DNA damage than UT mac (p<0.05) after pre- and simultaneous treatment, and similar protective effect in post-treatment (p>0.05).

In vivo

Bark (not specified)

Female W/Fu rats (n=10/group) were gavaged daily with **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) at the doses of 40 and 80 mg/kg for 8 consecutive weeks. Repair of DNA single strand breaks (SSB) and double strand breaks (DSB) 3 hours after 12 Gy whole body irradiation of rats were significantly improved in AE treated animals ($P<0.05$) (Sheng *et al.* 2000a).

Sheng *et al.* reported (2005) that **QA (Quinovic acid) esters** showed growth inhibition without cell death in HL-60 and HML cells *in vitro*, which would be stimulatory to DNA repair. *In vivo* 50 rats were divided into five groups: doxorubicin (DOX), **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) (80 mg/kg) + DOX, quinic acid (200 mg/kg) + DOX and ammonia-treated quinic acid (QAA) (200 mg/kg) + DOX and saline control. Peripheral blood WBC data indicated, that both QA and QAA could induce recovery from DOX induced leukopenia (7.1 $X10^{12}$WBC/L) about as effectively as 80 mg/kg AE (8.1, 8.5 and 8.4$X10^{12}$ WBC/L, respectively, $p<0.05$). Based on these data it was concluded that a QA analog was at least one class of active ingredients in AE.

Carboxy alkyl esters of *Uncaria tomentosa* (obtained from **a commercially available aqueous extract from the bark of *Uncaria tomentosa*-AE)** were reported to facilitate recovery (4 weeks) of sensorineural functions following exposure to a damaging level of noise. Long–Evans rats were divided into four treatment groups: vehicle-control, noise-only, AE-only and AE+noise. AE treatment facilitated almost complete recovery of outer hair cell function and limited the magnitude of cell loss. The loss of neural sensitivity to pure tone stimuli was inhibited with AE treatment (Guthrie *et al.* 2011).

Single substance

Pteropodine (100–600 mg/kg) was reported to significantly decrease the frequency of sister-chromatid exchanges and micronucleated polychromatic erythrocytes in mice administered with 10 mg/kg of doxorubicin. Furthermore, pteropodine partially corrected bone marrow cytotoxicity induced by doxorubicin, as it showed an improvement in the rate of polychromatic erythrocytes. The antigenotoxic effect of pteropodine was detected with all tested doses. The highest reduction of MNPE (micronucleated polychromatic erythrocytes) was achieved with 600 mg/kg of pteropodine at 24 hours (89%, $p\leq0.05$). Besides, 600 mg/kg of pteropodine increased 25.8% ($p\leq0.05$) of the production of lymphocytes over the control value along a 96-hours assay (Paniagua-Pérez *et al.* 2009).

Antioxidant activity

In vitro

There are numerous investigations concerning the antioxidant activity of *Uncaria tomentosa,* but only four studies were performed *in vivo.* Amongst the *in vitro* tests, mainly the aqueous extract of the bark of *Uncaria tomentosa* was investigated, but ethanolic (50, 70, 96%) extracts of the bark, leaves, aqueous and ethanolic extracts of the bark mixed with leafs and aqueous, dichloromethanic, methanolic extracts of the roots and stem bark were also studied. Different types of assays were carried out in order to measure general free radical, superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid, lipid peroxidation scavenger activity. Generally, the tested preparations had intermediary antioxidant activity (Gonçalves *et al.* 2005; Sandoval *et al.* 2000; Sandoval-Cachón *et al.* 1998; Sandoval *et al.* 2002; Pilarski *et al.* 2006; Bors *et al.* 2011; Bukowska *et al.* 2012; Shi *et al.* 2010 and 2013; Piscoya *et al.* 2001;Demarchelier *et al.* 1997; Garcia *et al.* 2012; Choi *et al.* 2002; Ostrakhovich *et al.* 1997 and 1998; Miller *et al.* 1999; Amaral *et al.* 2009).

In a study a correlation between the capacity of DPPH reduction and the proanthocyanidins content was verified (Gonçalves *et al.* 2005). On the other hand, the alkaloid content does not seem to have a role in the antioxidant effect, because in three different assay, *Uncaria guianensis* was more potent that *Uncaria tomentosa* ($p<0.01$), yet the total oxindole and pentacyclic alkaloid content of UT was 35 fold>UG (Sandoval *et al.* 2002).

In vivo

Bark (not specified)

The antioxidant effect of **70% ethanolic bark** extract of *Uncaria tomentosa* (BHE) was demonstrated in rats with Walker-256 cancer by Dreifuss *et al.* (2010). The *in vivo* oxidative stress parameters (Catalase (CAT), SOD and glutathione-S-transferase (GST)) were measured in liver and/or tumour tissues. The presence of W-256 tumour induced significant changes in all of hepatic enzymes. In the control group the mean activity of CAT was reduced by 79%, SOD increased by 252% and GST decreased by 59% when compared to baseline group. The treatment with BHE successfully normalised the activities of these enzymes. Regarding to CAT and GST, all tested doses (10, 50 and 100 mg/kg) of BHE significantly ($p<0.05$) increased its activities, drawing both enzymes to similar levels of those found in baseline condition. The activity of hepatic SOD was normalised only by the highest dose of BHE (100 mg/kg). CAT activity for the group treated with 10 mg/kg BHE had lower activity in tumour (59.9 ± 5.2 µmol·min·mg·protein^{-1}) than in liver (291 ± 17.8 µmol·min·mg·protein^{-1}), which is quite favourable. Like so, activity of tumour SOD was significantly reduced in all groups treated with BHE. In contrast, treatment with BHE did not lead to any significant differences in reduced glutathione levels (GSH) and lipid peroxidation (LPO) rates in the tumour tissue.

The antioxidant properties of a **70% ethanolic** (BHE) extract of **the bark** of *Uncaria tomentosa* and its **two fractions choroformic ($CHCl_3$) and n-butanolic (BuOH)** were evaluated and compared *in vitro* (DPPH) and *in vivo* in rats (n=4-6/group) with Walker-256 cancer. In the hepatic tissue of the animals of the control group (vehicle), the SOD level was increased (6.36 U SOD/mg of protein), the CAT level was decreased (179.5 mmol/min·mg of protein) when compared to baseline values (1.99 U SOD/mg of protein, 399.8 mmol/min·mg of protein). The $CHCl_3$ fraction generated same values as the control group. Treatment with the BHE extract increased these values ($p<0.001$ and $p<0.05$, respectively) to 10.16 U SOD/mg of protein ($p<0.001$) and the BuOH fraction exhibited a significant increase only in the SOD activity, to 8.8 U SOD/mg of protein ($p<0.001$). Inversely, in the tumour tissue, SOD activity in the control group was 10.5 U SOD/mg of protein; BHE extract and its BuOH fraction significantly reduced these levels Results in the tumours are in opposition of those found in the liver, but with much lower values of CAT. In the liver samples of the control group 28.22 nmol/mg of protein was the observed level of the LPO. BHE and BuOH group reduced significantly these levels, in an attempt to return this parameter to baseline levels. In the tumour the BHE extract and its BuOH fraction reduced LPO levels in a statistically significant manner, the $CHCl_3$ fraction did not alter this parameterin neither samples (Dreifuss *et al.* 2013).

The acute effects of *Uncaria tomentosa* (UT) (**70% ethanolic** extract of the **bark**, 2.57% pentacyclic oxindole alkaloids) in the recovery of neutrophils after chemotherapy-induced neutropenia were assessed. Ifosfamide-treated mice received oral doses of 5 and 15 mg of UT extract and intraperitoneal doses of 3 and 9 µg of filgrastim, respectively, for four days (6 mice/group). There were no differences in levels of non-protein thiols or in the activities of antioxidant enzyme catalases or superoxide dismutase (SOD) among *Uncaria tomentosa*, filgrastin, and control groups (Farias *et al.* 2011).

Unknown part

The renoprotective effects of a **dry extract** of *Uncaria tomentosa* (DUT, **part not specified**) were demonstrated on ischemic acute kidney injury (AKI) induced by renal clamping in rats (n=35). The groups were: Sham (no ischemia), ischemia (45 minutes), DUT +ischemia (rats treated with 20 mg DUT daily for 5 days prior to the simulation of ischemia), DUT + sham (rats treated with 20 mg DUT daily for 5 days). The ischemic animals presented reduction of the renal function (creatinine clearance) when compared to those in the sham group (0.17 ml/min vs. 0.54 ml/min, respectively, $p<0.05$), confirming the episode of ischemic AKI. The Ischemia+ DUT group presented attenuation in the reduction of creatinine clearance (0.54 ml/min vs. 0.17 ml/min, $p<0.05$) in comparison to the group that was not treated. The ischemia group presented an increase in the excretion of urinary peroxides when compared to the sham group (11 nmol/g vs. 3 nmol/g, respectively, $p<0.05$). In the ischemia+ DUT group there was a statistically significant decrease in the excretion of urinary peroxides, compared to the ischemia group (3.6 nmol/g vs. ±1.5 nmol/g respectively, $p<0.05$). The ischemia group presented a statistically significant increase in urinary TBARS when compared to the control group (281 nmol/g vs. 98 nmol/g, respectively, $p<0.05$). The values of lipid peroxidation (TBARS) were reduced in the group treated with DUT in comparison to the ischemia group (151 nmol/g vs. 281 nmol/g, respectively, $p<0.05$). The pre-treatment with DUT promoted a functional protection, which may be related to the antioxidant activities of the phytotherapic agent (Vattimo and da Silva, 2011).

Antibacterial and antifungal properties

Although *Uncaria tomentosa* is not used extensively to treat infections in traditional medicine, recently some *in vitro* reports evaluating antibacterial and antifungal activities were published. Both, extracts of the bark (80% ethanolic, aqueous, methanolic, dichlormethanic) and isolated constituents (isopteropodine, arthocamin A, 5'-hydroxycudraflavone A, dihydrocudraflavone B) were tested. In some bacterial (*B. cereus, B. subtilis, Enterococcus faecalis, Staph. aureus, Staph. epidermidis*, *E. coli, M. flavus, Strep. Mutans, Klebsiella pneumoniae*) and fungal strains (effective against *Candida albicans* in one out of three study) moderate effects was observed (White *et al.* 2011; Kloucek *et al.* 2005; García *et al.* 2005).

Antiviral effect

In two *in vitro* studies, the hydroalcoholic extracts of the stem bark exhibited antiherpetic activity (Herpes simplex virus type 1, HSV-1), by inhibiting the attachment of HSV-1; and significantly decreased Dengue virus-2-Ag in monocytes as well. The oxindole alkaloid purified fraction and quinovic acid glycosides purified fraction did not present activity against HSV1, yet the alkaloidal fraction was more effective than the extract in the case of DENV infection (Reis *et al.* 2008; Caon *et al.* 2014). The antiviral activity against Vesicular stomatitis virus was evident for six quinovic acid glycosides, isolated from *Uncaria tomentosa* (Aquino *et al.* 1989).

Alzheimer's disease

In vitro

Unknown part

A proprietary extract of the root or the inner bark of *Uncaria tomentosa* (**PE**) inhibited the formation of both type II diabetes and Alzheimer's disease amyloidosis *in vitro,* as measured by thioflavin T fluorometry assay. Dose-dependent inhibition of β-amyloid fibril growth and formation was shown when the extract was found also to inhibit β-amyloid-β-amyloid interactions in solid phase binding assay and exhibited a dose-dependent amyloid-dissolving activity against performed Alzheimer's

disease amyloid fibrils, with low doses dissolving the fibrils by ~70% in 2 hours of incubation. Similarly, islet amyloid fibrils or islet amyloid polypeptide were dissolved by more than 80% in the same time period from exposure to the extract (McKenna *et al.* 2002). In another study mitraphylline was shown to bind with β-amyloid 1-40 protein (Frąckowiak *et al.* 2006)

In vivo

Unknown part

A modified animal model was used to determine the efficacy of a proprietary extract of the root or the inner bark of *Uncaria tomentosa* PE on disruption of pre-deposited Aβ 1-42 amyloid fibril deposits in brain. Adult Sprague-Dawley rats (n=9/group) were stereotaxically cannulated into the hippocampus and infused for three days with 62.5μM Aβ 1-42 (25 μg total), followed by four days of no treatment. Then the animals were infused for seven days with either distilled water or PE at an Aβ:PE ratio of 1:5. Animals infused with Aβ 1-42 alone followed by distilled water, had Congo red scores of 1.78, whereas animals infused with Aβ 1-42 followed later with PE had Congo red scores of 0.64. Therefore, a significant ($p<0.01$) 64% disruption/dissolution of pre-deposited Aβ 1-42 amyloid fibrils was observed following infusion of PE in comparison to controls (Cummings *et al.* 2000).

An assay guided fractionation and HPLC were used to isolate, characterise and test water-soluble active ingredients within a proprietary extract of the root or the inner bark of *Uncaria tomentosa* (PE) that possess potent Aβ fibrillogenesis inhibitory activity. The mixture of seven major water-soluble ingredients (7C) were purified and tested in various assays to determine their efficacy in comparison to PE and to major alkaloids present in *Uncaria tomentosa*. 7C was a more potent inhibitor of Aβ fibrillogenesis than PE alone. In one study, incubation of PE with Aβ 1-42 for 1 day (1:1 weight ratio) caused a 53.2% disruption of Aβ fibrils, whereas 7C resulted in a significant 87.3% inhibition. In a rodent model 1-week co-infusion of PE+Aβ 1-42 into hippocampus led to a 51.0% inhibition of Aβ fibril deposition into brain, whereas 7C+Aβ 1-42 resulted in a significant 89.2% inhibition. Amyloid inhibitory effects were not observed using purified alkaloids derived from Cat's claw (Snow *et al.* 2000).

A group of major water-soluble components isolated from *Uncaria tomentosa* (7C), caused a marked reduction of amyloid plaque burden in a transgenic animal model of Alzheimer's disease. Two groups (n=6 for saline-treated; n=5 for 7C treated) of 6-8 month old, plaque-producing transgenic mice were stereotaxically infused for 2 weeks (with osmotic pumps) into cortex with saline or 7C. Quantitative analysis of brain sections revealed that 7C caused a 48.4% reduction in % amyloid burden, a 78.3% reduction in amyloid plaque number, and a 15.3% reduction in plaque diameter (Snow *et al.* 2001).

In a rat model of Alzheimer's disease β-amyloid deposition, a proprietary extract of the root or the inner bark of *Uncaria tomentosa* (PE), administered by directing infusion into rat hippocampus caused a significant 74% inhibition of β-amyloid deposition ($p<0.01$) from a dose of 1 μl, and 87% inhibition from a dose of 10 μl, each applied for seven days. Following the treatment the cellular architecture of hippocampal brain sections showed no obvious adverse effects. In another study, the *in vitro* β-amyloid formation-inhibiting, growth-inhibiting, and performed β-amyloid-dissolving activity of cat's claw extract was markedly enhanced by the addition of a standardised *Ginkgo biloba* extract (McKenna *et al.* 2002)

Other *CNS related activities*

In vitro

Mainly single compounds were tested *in vitro* for their CNS related activities (neuroprotection, Parkinson's disease). Neuroprotective activity was shown for isorhynchophylline, isocorynoxeine,

hirsuteine and hirsutine against glutamate-induced cell-death (Shimada *et al.* 1999). Specific water-soluble components derived from *Uncaria tomentosa*, yielded in a previous study (Snow *et al.* 2000) inhibited apha-synuclein (using NAC-P) fibrillogenesis (Castillo *et al.* 2001). Additionally, rhynchophylline and isorhynchophylline were reported to be noncompetitive antagonists of the NMDA receptor, pteropodine and isopteropodine act as positive modulators of muscarinic M_1 and 5-HT_2 receptors, and corynantheine, dihydrocorynanthe, geissoschizine methyl ether were found to be partial agonists for 5-HT receptors (Kang *et al.* 2002a, 2002b, Kantani *et al.* 1985).

Two studies investigated the neuroprotective_antioxidant_effects of two commercially_available_aqueous extracts (AE, NAE). Both extracts had protective effect against 6-OHDA induced cell damage; AE could reduce the aggregation of α-synuclein (Shi *et al.* 2010, 2013)

In vivo

Unknown part

Bigliani *et al.* (2013) examined the effect of orally administered *Uncaria tomentosa* decoction (UTE, part not specified) during 7, 15, 30 and 90 days of treatment on the expression of anxiety, as expressed in the elevated plus maze test in male Albino Swiss mice. UTE revealed an anxiogenic effect in relation to the control group at 15 and 30 days, but it was reversed after 90 days of administration. Thus UTE have a transient anxiogenic effect.

Single substances

Mohamed *et al.* (2000) reported, that the total alkaloids of *Uncaria tomentosa* had a beneficial effect on memory impairment induced by the dysfunction of cholinergic systems in the brain of mice (n=18/group); the effect of total alkaloids is partly attributed to the oxindole alkaloids tested. *Uncaria tomentosa* (**plant part not specified**, powdered commercial product) extract was used to yield the alkaloids Uncarine E, Uncarine C, mitraphylline, isorhynchophylline and rhynchophylline. The total alkaloids (10-20 mg/kg, *i.p.*), the single alkaloids (10-40 mg/kg, i.p.) as well as the muscarinic receptor agonist oxotremorine (0.01 mg/kg, i.p.) significantly attenuated the deficit in retention performance induced by the muscarinic receptor antagonist scopolamine. The effective doses of uncarine C and mitraphylline were larger than those of other alkaloid components. Uncarine E (20 mg/kg, i.p.) also blocked the impairment of passive avoidance performance caused by the nicotinic receptor antagonist CPP, but it failed to affect the deficit caused by the benzodiazepine receptor agonist diazepam. Rhinchophylline significantly reduced the mecamylamine-induced deficit in passive avoidance behaviour, but it failed to attenuate the effects of CPP and diazepam.

Rhynchophylline (Rhy) reduced the spontaneous motor activity and enhanced the sedative and hypnotic effects of sodium pentobarbital in mice. Twentyone mice were divided into three groups: Group A (*i.p.* saline), Group B (*i.p.* sodium pentobarbital (SP), 35 mg/kg) and group C (*i.p.* SP 35 mg/kg and Rhy 10 mg/kg). The righting reflex was normal in group A, while all mice in groups B and C lost their reflex for 13 and 51 minutes, respectively (p<001). Another 28 mice were randomly divided into four groups and injected *i.p.* SP 20 mg/kg, SP plus Rhy 20 or 40 mg/kg, and only Rhy 40 mg/kg, respectively. In 2 hours the righting reflex was retained in all groups but in group C five out seven mice lost their reflex (p<001). 20 mg/kg *i.p.* Rhy (n=10) caused a reduction in the number of motor activity (from 17 to 6 times/min, p<0.01), compared to control (saline, n=10). Rhy *in vivo* (20 and 40 mg/kg) also modulated the neuro-transmitter metabolism: increased the 5-HT content in the hypothalamus and cortex, but reduced the DA concentrations in the cortex, amygdala, and spinal cord. Rhy *in vitro* (3 and 30 μM) promoted the release of endogenous DA from 4 brain regions. The release of 5-HT was increased in 2 brain regions and decreased in hypothalamus slice. However, Rhy inhibited the release of both 5-HT and DA evoked by high potassium (Shi *et al.* 1993).

A fraction of *Uncaria tomentosa* (speciophylline, uncarine F, mitraphylline, isomitraphylline, pteropodine, rhynchophylline, isorhynchophylline and isopteropodine, accounting for 95% of the sample) was used to characterise antinociceptive activity in chemical (acetic acid-induced abdominal writhing, formalin and capsaicin tests) and thermal (tail-flick and hot-plate tests) models of nociception in mice. *Uncaria tomentosa* (UT) fraction given by the *i.p*. route dose-dependently suppressed the behavioural response to the chemical stimuli ($p<0.0001$) in the models indicated, and increased latencies in the thermal stimuli models. The antinociception caused by UT fraction in the formalin test was significantly ($p<0.0001$) attenuated by *i.p*. treatment of mice with ketanserin (5-HT2 receptor antagonist), but was not affected by naltrexone, atropine), L-arginine, prazosin, yohimbine or reserpine. Together, these results indicate that UT fraction produces dose-related antinociception in several models of chemical and thermal pain through mechanisms that involve an interaction with 5-HT2 receptors (Jürgensen *et al.* 2005).

Isorhynchophylline (100 mg/kg, $p<0.05$) significantly depressed total activity of locomotor activity in male ICR mice with and without excitation, elicited with methamphetamine, measured by home cage activity apparatus (Sakakibara *et al.* 1999).

Endometriosis, effects on oestrogen, progesterone

In vitro

Bark (not specified)

The aqueous solution of the bark of *Uncaria tomentosa* (SUT) was suggested to interact with distinct binding sites on the oestrogen receptor (ER). A significant reduction of [^{3}H] oestradiol-specific binding with SUT (10 and 20 µg) was detected following 1 h of exposure to the drug. When the cytosol was pre-treated with SUT or tamoxifen (TMX) before addition of [^{3}H] oestradiol, specific binding was reduced by 47.2% and 69.3%, respectively. Increasing concentrations of [^{3}H] oestradiol were incubated with cytosol of hormone dependent tumours in the presence or absence of fixed concentrations (10 and 20 µg) of SUT. SUT was clearly a non-competitive inhibitor of [^{3}H] oestradiol binding (Salazar and Jayme 1998).

In vivo

Unknown part

Nogueira Neto *et al.* (2011a) reported histological changes in parenchyma's epithelial layer of the uterus and ovarian of rats (n=29) with experimental endometriosis treated with *Uncaria tomentosa* extract which were comparable with contraception. UT group received gavage with 32 mg/ml of *Uncaria tomentosa* **aqueous extract** (commercial product), 1 ml administered daily and the placebo group received 1 ml of saline 0.9% per day, during for 14 days (both groups); the leuprolide group received leuprolide acetate 1 mg/kg bodyweight applied single subcutaneous dose. In the 15th day of UT group presented nine samples (90%) with immature ovarian follicles, whereas the placebo group did not present any case and in the leuprolide group there were eight rats (88%) with the same change. The placebo group showed mature corpus luteum in all animals, occurring less frequent in UT (10%) and leuprolide (22%) groups. The uterine epithelium showed weak proliferative in nine (90%) samples of the UT group, in two (20%) animals in the placebo group and seven (77.8%) rats in the leuprolide group. 160 tablets of 100 mg were grinded in a porcelain mortar dissolved in 500 ml 0.9% NaCl.

Nogueira Neto *et al.* (2011b) also investigated the influence of *Uncaria tomentosa* on experimentally induced endometriosis. Wistar rats (n=24) with experimental endometriosis were divided into two

groups. Group UT received *Uncaria tomentosa* extract orally (32 mg/day), and group C (control group) received a 0.9% sodium chloride solution orally (1 ml/100 g of bodyweight/day). Both groups were treated with gavage for 14 days. There was a significant increase (p=0.01) between the initial and final average volumes of the autotransplants (endometriosis implant) in the control group, while the treatment with *Uncaria tomentosa* caused a marked reduction in the growth over time (p=0.009). Histologically, in the experimental group (n = 10) six rats had a well-preserved epithelial layer, three had mildly preserved epithelium, and one had poorly preserved epithelium. The control group (n = 12) presented seven cases (58.3%) of well-preserved epithelial cells and five cases (41.7%) of mildly preserved epithelial cells.

It was reported that in human granulosa cells obtained from patients undergoing *in vitro* fertilisation, *Uncaria tomentosa* extracts **(part not specified)** at amounts equivalent to dietary doses, dose-dependently (100 pg/ml to 10 µg/ml) caused statistically significant inhibition of progesterone production. Administered orally to sexually mature female rats for eight weeks, a low dose of cat's claw extract (2 mg/day), equivalent to the manufacturer's suggested daily dosage, produced no significant changes in profiles of electrolytes, kidney, liver or glucose. For six weeks no changes were noticed in circulating hormone levels compared to the untreated controls. By the eighth week however, serum progesterone and oestradiol levels were reduced by 68% and 71%, respectively, in both the high dose group (20 mg/day) and a low dose group (2 mg/day) (McKenna *et al.* 2002).

Cardiovascular effects

Single substances

In vitro

Mainly single substances were tested *in vitro* for cardiovascular effects. Isorhynchophylline inhibited the automaticity and contractile force of isolated guinea pig atrium, rhynchophylline caused concentration dependent inhibition of platelet aggregation induced by arachidonic acid, collagen and ADP and hirsutine exhibited calcium channel blocking activity mainly through competitive inhibition of the voltage dependent calcium influx Less potently rhynchophylline and isorhynchophylline were found to inhibit the voltage dependent calcium channels as well (Chen *et al.* 1992; Yano *et al.* 1991;. Zhang *et al.* 1987; Yamahara *et al.* 1987; Zhu *et al.* 1995).

In vivo

In several performed *in vivo* studies rhynchophylline (Rhy) and isorhynchophylline (iRhy) were investigated. Rhy inhibited venous thrombosis in rats and it prevented both ADP and collagen plus adrenaline induced thrombotic death. Rhy also reduced the mean arterial pressure, heart rate, renal blood flow and coronary blood flow in dogs (Chen *et al.* 1992; Jin *et al.* 1991; Shi *et al.* 1992). IRhy was able to reduce the mean arterial flow as well, but not the renal blood flow. It lowered the systolic arterial blood pressure, diastolic arterial blood pressure and heart rate in normo-, and hypertensive rats and dogs (Shi *et al.* 1989; Shi *et al.* 1992). In a further study iRhy was inhibited the calcium currents in rats and guinea pigs (Gan *et al.* 2011*)*

3.1.3. Safety pharmacology

No studies are available.

3.1.4. Pharmacodynamic interactions

Moreno *et al.* (2007) treated male Wistar rats were treated with an aqueous extract of *Uncaria tomentosa* (32 mg/ml n=5) or 0.9% NaCl solution (control, n= 5) for 7 days. After this period, $Na^{99m}TcO_4$ (3.7 MBq, 0.3 ml) was injected through the ocular plexus and after 10 minutes the rats were killed. There were significant ($p<0.05$) alterations in $Na^{99m}TcO_4$ uptake between the control and the treatment group in the heart (0.57 vs. 0.39% ATI/organ, $p<0.05$), in the pancreas (0.07 to 0.19% ATI/g, $p<0.05$) and in the muscle (0.07 to 0.18% ATI/g $p<0.05$) after treatment with this extract.

Quílez *et al.* (2012) investigated changes in the pharmacological effect of diazepam (2 mg/kg) on the CNS when administered together with a commercial product of *Uncaria tomentosa* (PT), (7.14 mg/kg and 3.54 mg/kg). PT, at both doses, enhanced the action of diazepam on spontaneous motor activity ($p<0.001$) and, at the lower dose, exploratory ability. PT showed only a slight decrease (19.7%) on muscular relaxation effect of the benzodiazepine.

3.1.5. Conclusions

The pharmacological data is quite diverse on *Uncaria tomentosa*, concerning the used extracts and biological properties tested. The main concern is, that most of the studies indicate 'bark' for the drug used, without specifying whether it is obtained from the root or the stem. From the 35 studies in the primary pharmacodynamics section 19 studies mentioned just 'bark', 7 studies didn't state the part used and only 5 and 3 studies specified stem bark and root bark, respectively. A number of investigations used commercial products, without adequate information on the contained drug/extract. Thus, it is difficult to draw clear conclusion from the data.

In the *in vivo* studies, the administered dose ranged between 5 and 2000 mg extract/kg bodyweight, most frequently it ranged from 50-500 mg extract/kg bodyweight.

All of the extracts comprised by the preclinical data were reported to have pleiotropic anti-inflammatory or immunmodulatory activity.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

After oral administration of isorhynchophylline (ISOR) to rats (37.5 mg/kg) ISOR was identified in plasma, bile, urine and faeces. 11-Hydroxyisorhynchophylline 11-O-β-D-glucuronide (MI1) and 10-hydroxyisorhynchophylline 10-O-β-D-glucuronide (MI2) were found in bile, and free 11-hydroxyisorhynchophylline (MI3) and 10-hydroxyisorhynchophylline (MI4) were found in urine and faeces. Within 24 hours, 71.6% of ISOR was found in the faeces and 13.8% in the urine. Monitoring by LC-MS showed that 8.5% of ISOR was metabolised to MI3 and MI4 in a ratio of ca. 1:1. Specific inhibition of CYP isoenzymes indicated that CYP2D, CYP1A1/2 and CYP2C participate in ISOR hydroxylation (Wang *et al.* 2010).

Pharmacokinetic interactions

The inhibitory activity of the decoction of the bark of *Uncaria tomentosa* (conc.: 1.56-100 mg/ml) for cytochrom P450 3A4 was measured *in vitro.* The IC_{50} of the decoction was 46.8 mg/ml (Budzinski *et al.* 2008). Yet, in another study, *Uncaria tomentosa* tincture was found to be an inhibitor, it gave inhibition readings>100% (saturation of the enzyme) for relative concentrations >3.13% (IC_{50} :0.79 relative concentration). However, the strength of the inhibition is a questionably significant effect compared to typical CYP 3A4 inhibitors (Budzinski, 2000).

In another study, the inhibitory effects of alcoholic extract of *Uncaria tomentosa* on CYP2D6, CYP2C9, and CYP2C19 were minor (in order of decreasing potency) (Foster *et al.* 2003).

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

The oral single dose LD_{50} could not be calculated because at the maximum concentration of the various *Uncaria tomentosa* preparations, no lethal effect had been observed. However, it was concluded that the LD_{50} of **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) was greater than 8 g/kg since no single death in the treatment groups had been observed at this dose or below it. The LD_{50} of two other preparations, a bark powder (commercial product) and a water: ethanol extract (4% alkaloids) of *Uncaria tomentosa* were observed to be greater than 2 and 5 g/kg, respectively. The MTD for AE was also greater than 8 g/kg, while it was greater than 2 g/kg for the bark powder and between 2.5 and 5.0 g/kg for the water: ethanol extract (McKenna *et al.* 2002).

Keplinger *et al.* (1999) performed an acute oral toxicity study in mice (n=10). The subjects were treated with freeze dried aqueous root extract – containing 35 mg total pentacyclic oxindole alkaloids per g; 6% yield from crude drug. Signs of reaction to treatment, observed shortly after dosing, consisted of lethargy and piloerection. Death of two out of ten mice occurred within 4 hours of treatment. Autopsy revealed haemorrhage of the stomach and intestines, and pallor of the liver and spleen. Recovery of survivors, as judged by external appearance and behaviour, was apparently complete within five days of treatment. This observation was substantiated by normal bodyweight gains, compared with controls and normal autopsy findings. The acute median lethal dose (LD_{50}) to mice of freeze-dried aqueous root was found to be greater than 16 g/kg bodyweight**.**

Consistent with these results, Valerio and Gonzales (2005) tested mice using the cat's claw aqueous extract at a dose of 5 g/kg bodyweight and intraperitoneal administration of a dose of 2 g/kg bodyweight reported no apparent toxicity.

3.3.2. Repeat dose toxicity

Sheng *et al.* (2000a) did not find acute toxic signs or symptoms in an acute toxicity study, performed on rats in the repeated daily supplement experiments. All groups of the rats gained weight during supplementation while no bodyweight differences were found among the groups at any time point for **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) at 40 and 80 mg/kg for 8 weeks and 160 mg/kg for 4 weeks. While no evidence of toxicity was observed with AE, there was a statistically significant reduction in bodyweight ($P<0.05$) and food consumption ($P<0.01$) in rats receiving the same dose of another water extract (Commercial extract A, water soluble 4:1 *Uncaria tomentosa* extract, but not purified from high molecular weight toxic components). The histopathological examination revealed no increased pathological changes such as necrosis, fibrosis, increased mitotic activity and proliferation when compared with control rats.

Keplinger *et al.* (1999) performed a 4-week oral toxicity study in rats. The aqueous acid extract (containing 7.5 mg total oxindole alkaloids per g and 300 mg sodium chloride per g as remnant from neutralisation; 10% yield from crude drug) was used in the 4-week study. A daily dose of 1000 mg/kg per day for 28 days caused a slight but statistically significant increase in percentage of lymphocytes and decrease in percentage of neutrophil granulocytes and, in addition, an increase of the relative weight of the kidneys in rats of both sexes. Since the histology of the kidneys was normal, there is no

explanation for this finding. There were no differences between the test group and the control group with all other respects. There were no mortalities during the study.

3.3.3. Genotoxicity

Genotoxicity and antigenotoxicity

The aqueous (boiling water) extract of *Uncaria tomentosa* (drug not specified) was assayed for anti-genotoxicity using the Somatic Mutation And Recombination Test (SMART) in *Drosophila melanogaster*. Hydrogen peroxide was used as an oxidative genotoxicant to test the anti-genotoxic potency. The extract did not show a significant genotoxicity at concentrations tested (0.5-1.9 mg/ml) (Romero-Jiménez *et al.* 2005).

Both uncarine C and F showed weak activity (IC_{12}: 140 and 120 µg/ml, respectively) against the RS321 and RS322 yeast strains. For comparison: the known active agent, streptonigrin has an IC_{12} value of 0.65 µg/ml against the RS321 strain, and 0.4 µg/ml against the RS322 strain while the topoisomerase I inhibitor camptothecin had IC_{12} values of >20 and 0.6 µg/ml against the same two strains (Lee *et al.* 1999).

The mutagenic potential of extracts (petroleum ether, chloroform, chloroform:methanol 9:1, methanol, water) and chromatographic fractions (Sephadex LH-20 of chloroform/methanol extract) of *Uncaria tomentosa* bark was tested at 10, 50, 75, and 100 µg/plate in Salmonella/mammalian microsome TA 98, TA 100, TA 1535, TA 1537 and TA 1538 strains of *Salmonella typhimurium*, with and without metabolic activation from Aroclor 154-induced rat liver homogenate. Cat's claw did not show evidence of mutagenicity with or without metabolic activation (Rizzi *et al.* 1993).

3.3.4. Carcinogenicity

No studies are available.

3.3.5. Reproductive and developmental toxicity

Ten g of the bark of *Uncaria tomentosa* was extracted with 50 ml of water for 12 hours in 37°C and its effect were evaluated by application of the chicken embryo model. Among three groups of eggs (n=360), two were injected with different doses of the extract (0.0492 mg/200 µl for group 1 and 0.492 mg/200 µl for group 2). To the third control group 200 µl of physiological salt was applied. Significant differences were observed for mean corpuscular volume of erythrocytes (MCV, P=0.002), mean cell haemoglobin concentration (MCHC, P=0.00001) and mean amount of cell haemoglobin (MCH, P=0.02). Cat's claw administration disturbed embryogenesis in both doses. In the first group it increased the MCV and decreased the MCHC. In the second it decreased both MCH and MCHC (Pilarski *et al.* 2009).

Cat's claw extract (2 mg/day or 20 mg/day p.o., no further data available) was administered orally to sexually mature female rats for eight weeks. For six weeks no changes were noticed in circulating hormone levels compared to the untreated controls. By the eighth week however, serum progesterone and oestradiol levels were reduced by 68% and 71%, respectively, in both the high dose group and a low dose group. It was concluded that these results indicate, that *Uncaria tomentosa* has the potential to selectively alter ovarian hormone production and therefore should be used with care. In a patent, mice administered an aqueous extract of the root in their drinking water (6.25 mg/kg and 25 mg/kg, *per os*) showed no signs of toxicity; however, the female mice, when caged with male mice produced no offspring (McKenna *et al.* 2002).

3.3.6. Local tolerance

No studies are available

3.3.7. Other special studies

In vitro toxicity studies

An erythrocyte suspension was incubated with four extracts (5-500 µg/ml) of *Uncaria tomentosa*: ethanol extract of the bark, leaves, aqueous extract of the bark and leaves were incubated with erythrocytes. One gram of the raw material (bark and leaves) was extracted with 10 ml of water or 96% ethanol at 37 °C for 8 hours, then centrifuged and dried. At these conditions they recovered about 111 mg extract/g of bark and about 158 mg/g of leaves. Extraction with 96% ethanol resulted 123 mg extract/g of bark and 158 mg extract/g of leaves. It was found that no extract of *Uncaria tomentosa* induced haemolysis. However, a slight, statistically significant decrease in haemoglobin outflow was observed in the erythrocytes incubated with ethanol extracts from the concentration of 100 µg/ml, and the cells incubated with aqueous extracts from the concentration of 250 µg/ml (Bors *et al.* 2011).

Bors *et al.* (2012b) also reported that ethanolic and aqueous extracts (100 µg/ml and 250 µg/ml, respectively) from the leaves and bark of *Uncaria tomentosa* caused changes in the size and shape of the erythrocytes. Externalisation of phosphatidylserine on the erythrocytic surfaces was also noticed during incubation with extracts at a concentration of 250 µg/ml. Four extracts (5-500 µg/ml) of *Uncaria tomentosa* (**ethanol extract of the bark, leaves, aqueous extract of the bark and leaves)** were incubated with erythrocytes. Preparement of the extracts can be seen above (Bors *et al.* 2011). All extracts induced changes in the erythrocyte membrane properties, whereas ethanolic extracts from bark induced the most significant changes. An increase in red blood cells volume and changes in the erythrocytes shape were observed from the concentration of 100 µg/ml for ethanol leaf extract ($p<0.001$) and ethanol bark extract ($p<0.001$) as well as from 250 µg/ml for aqueous leaf extract ($p<0.001$) and aqueous bark extract ($p<0.001$).

In a third study, Bors *et al.* (2012a) observed oxidative changes in mononuclear blood cells exposed to both ethanol and aqueous extracts obtained from *Uncaria tomentosa* bark and leaves (extraction see above, at Bors *et al.* 2011). In the cells studied the extracts induced apoptosis and a decrease in viability of mononuclear blood cells, with the exception of the aqueous extract of leaves. In the cell viability studies, the strongest changes were observed for the ethanol extract of bark, which decreased the cell viability to 51.5% at 250 µg/ml. A statistically significant decrease in blood mononuclear cells viability was noted for the ethanol extract of leaves (to 69.6%) and aqueous extract of bark (to 87.6%) only at their highest concentration of 250 µg/ml. IC_{50} was over 250 µg/ml for all extract studied. No statistically significant changes in the cell size were observed both for aqueous extracts of leaves and bark. A decrease in the cell size provoked by ethanol extract of bark was observed at 100 mg/ml, whereas for ethanol extract of leaves it was noticed at 250 µg/ml. Changes in the blood mononuclear cell granularity were observed at 250 µg/ml for all extracts examined. The strongest changes were observed for the ethanol extract of bark, which increased cell granularity at 50 µg/ml and changed cell size at 100 µg/ml.

Bukowska *et al.* (2012) showed, that ethanolic and aqueous extracts from leaves and bark of *Uncaria tomentosa* (preparation see at Bors *et al.* 2011) at the concentration of 250 µg/ml were not toxic to erythrocyte catalase because they did not induced a statistically significant change in catalase activity after 1 h ($p>0.05$) and 5 hours ($p>0.05$) of incubation (Bukowska *et al.* 2012).

An aqueous extract of the dried bark of *Uncaria tomentosa* was not cytotoxic as measured by tryptan blue exclusion to epithelial (HT29) and macrophage (RAW 264.7) cells when treated at a concentration of 25-200 µg extract/ml overnight. Viability of treated cells was >90% and not significantly different than control untreated cells. The extract was prepared with boiling water (20 g/L) for 30 minutes and then was left overnight in room temperature, and then it was filtered and diluted to 5 mg/ml (Sandoval-Chachón *et al.* 1998).

An aqueous extract of *Uncaria tomentosa* (500 mg/ml) was analysed for the presence of toxic compounds in Chinese hamster ovary cells (CHO) and bacteria (*Photobacterium phosphoreum*). Toxicity was evaluated by natural red assay (NR), total protein content (KB), tetrazolium assay (MTT) and Microtox test. The extracts of *Uncaria tomentosa* did not show toxicity *in vitro* at the concentrations tested (10, 20, 30, 40, 50, 75 and 100 mg/ml) (Santa Maria *et al.* 1997)

Leukemic cells were taken from 53 children with acute leukaemia and four cell lines, Jurkat, CCRFCEM, HL-60, and K-562 were tested for sensitivity to *Uncaria tomentosa* (commercial product) by MTT assay, cell-cycle analysis and annexin-V binding assay. As the commercially available extract was without given concentration, for the purpose of this study, instead of their concentration, dilution range from 1:5 to 1:5,120 was used. Leukemic cells showed high resistance to the extract in all performed assays. Additionally, tested remedies stimulated survival of leukemic cells in 45 (96%) cases, while no effect was observed in normal lymphocytes (Styczynski and Wysocki, 2006).

3.3.8. Conclusions

Although there are limited toxicological data of *Uncaria tomentosa*, the results of non-clinical trials raise no safety concern.

Adequate tests on reproductive toxicity, carcinogenicity or genotoxicity have not been published.

3.4. Overall conclusions on non-clinical data

The pharmacological data is quite diverse on *Uncaria tomentosa*, concerning the used extracts and biological properties tested. The main concern is, that most of the studies indicate ‘bark’ for the drug used, without specifying whether it is root or stem. From the 35 studies in the primary pharmacodynamics section 19 studies mentioned just ‘bark’, 7 studies didn’t state the part used and only 5 and 3 studies specified stem bark and root bark, respectively. A number of investigations used commercial products, without adequate information on the contained drug/extract. Thus, it is difficult to draw clear conclusion from the data. Furthermore different extracts were tested in the preclinical studies.

In the *in vivo* studies, the administered dose ranged between 5 and 2000 mg extract/kg bodyweight, most frequently it ranged from 50-500 mg extract/kg bodyweight.

Specific data on pharmacokinetics, interactions and non-clinical information on the safety of *Uncaria tomentosa* are scarce.

Oral administration of the cortex of *Uncaria tomentosa* can be regarded as safe at traditionally used doses.

Adequate tests on reproductive toxicity, carcinogenicity or genotoxicity have not been published.

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No relevant data available.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No relevant data available.

4.2. Clinical efficacy

4.2.1. Dose response studies

Antimutagenic activity (DNA-repair), immunostimulant

A placebo-controlled study was carried out to compare two different doses of **a commercially available filtered aqueous extract from the bark (not specified) of *Uncaria tomentosa*** (AE), standardised to 8–10% CAEs (carboxy alkyl esthers) and almost free from oxindole alkaloids (≤0.05%) versus placebo by Sheng *et al.* (2001). Twelve healthy adults were randomly assigned into 3 groups and baselined for 3 weeks, and then supplemented AE at doses of 250 mg or 350 mg for 8 weeks. DNA repair after induction of DNA damage on monoclonal leukocytes by a standard dose of hydrogen peroxide was measured 3 times before supplement and 3 times after the supplement in the last 3 weeks of the supplement period. DNA damage did not change significantly before AE supplementation or after the supplementation (week 6, week 7), just blood sampling point at week 8 showed a significant difference between control and the group supplemented with AE at 350 mg/day.

There were significant increases of DNA repair after supplementation (week 6, 7, 8) by 12 and 15% (250 and 350 mg/day AE respectively), but there was no significant difference between the two doses used ($p<0.05$). There was an increased tendency of PHA (phytohemagglutinin) induced lymphocyte proliferation in the treatment groups, but it didn't reach the level of significance. In the case of staphylococcal enterotoxin superantigen (SEA) induced lymphocyte proliferation the SEA 0.001/ SEA 0.01 µg/ml proliferation index was significantly increased after the 8 weeks period in the 350 mg/day AE supplement group ($p<0.05$). That means an increase of lymphocyte proliferation stimulated by SEA at 0.001µg/ml or a decrease of lymphocyte proliferation when stimulated by SEA at 0.01 µg/ml (higher dose), when compared to control levels. No adverse effects, in terms of symptoms and signs, whole blood haematological analysis, or blood chemistry analysis have been found.

4.2.2. Clinical studies (case studies and clinical trials)

Rheumatoid arthritis

Forty patients with rheumatoid arthritis undergoing sulfasalazine or hydroxychloroquine treatment were enrolled in a randomised 52 weeks 2 phase study (Mur *et al.* 2002). During the first phase (24 weeks double blind, placebo controlled) patients were treated with a commercial product (HE) (20 mg aqueous acid-extracted dry extract from Uncariae tomentosae radix/capsule, 14.7 mg/g POA, no TOA) (HE group, n=19) or placebo (n=18). Patients were assigned to take 3 capsules daily (60 mg

Uncaria tomentosa extract). In the second phase (28 weeks) all patients received the plant extract. Morning stiffness was measured on a 5-step scale. At the end of the first phase the HE treatment resulted in a reduction of the number of painful joints compared to placebo by 53.2% vs. 24.1% p=0.044. There was also a reduction in the number of tender joints (50.65% vs. 24.1%, p=0.001), Ritchie index (49.1% vs.12.5%, p=0.002), and duration of morning stiffness (47.8% vs. 21.7%, p=0.002) compared to placebo. No changes were detected for the number of swollen joints, patient assessment of disease activity, subjective assessment of pain and laboratory variables except for an increase in the level of rheumatoid factor in the placebo group (p=0.041). In the second phase further intake of HE resulted in a reduced number of tender joints (67.5%, p<0.001), Ritchie Index (56.4%, p=0.001), and duration of morning stiffness (34.78%, p=0.004) compared to baseline values. Patients receiving HE only during phase 2, experienced a reduction in the number of tender joints (57.1%, p=0.003), swollen joints (44.6%, p=0.007) and the Ritchie Index (57.1% (p=0.044) compared to the values after 24 weeks of placebo treatment. NSAID drugs and prednisolone up to 10 mg/day or equivalent were permitted. During the first phase, adverse events occurred in 12 patients of each group. One patient taking HE withdrew from the study owing to gastritis and one patient from the placebo group because of diarrhoea. One patient from the HE group withdrew because of inefficacy. In the second phase, 7 other side effects were seen; none could be clearly attributed to the drug intake. No major side effects were seen in the active and the placebo group.

Castañeda *et al.* (1998) conducted a placebo controlled, randomised, multicentre study on patients with rheumatoid arthritis and evaluated (n = 60) the therapeutic potential of an *Uncaria tomentosa* product: 3X2 capsules/day 150 mg *Uncaria tomentosa* extract/capsule (no further details discussed). It was administered for 6 months. There was no information about the ingredients of the product. Clinical and laboratory measurements were performed every four weeks, evaluating morning stiffness in minutes, daytime and night-time pain in a 0-10 scale, number of painful joints, number of swollen joints, functional capacity in 1-4 scale, sedimentation rate. All the investigated variants showed statistically significant differences compared to placebo at the end of the 6 months. Morning stiffness was reduced by 65.5%, but in the placebo group it increased 22.36% (p<0.01). The daytime intensity of pain reduced by 48.3% in the treatment group, while in the placebo group by 14.3% (p<0.001). Night-time intensity of pain showed the same results (57.8% vs. 12.7% reduction in the treatment vs. placebo group, p<0.01). The number of painful and swollen joints reduced 70.59% and 65.3% respectively in the group received *Uncaria tomentosa* capsule, while these values in the placebo group were 9.4% and 2.4%, respectively (p<0.001 for both). Laboratory parameters also had significant changes, the sedimentation rate, which is elevated during inflammation reduced 46.3% in the *Uncaria tomentosa* group, while in the placebo group the reduction was 6.12% (P<0.05) and the hematocrit was elevated in the treatment but reduced in the placebo group by 5.4% and 10.76%, respectively (p<0.05). There were no statistical differences between the experienced adverse effects. Ten subjects dropped out, two of them because of adverse events. 25.8% of the treatment group and 13.8% of the control group had adverse reactions, most frequently gastric disturbances. Other adverse reactions were: nausea, diarrhoea, bitter mouth, meteorism and constipation. There was no statistically significant difference between the two groups in regard to the occurrence of the adverse reactions (p=0.245).

Table 5: Clinical studies on humans, in Rheumatoid arthritis

Abbreviations: PC=placebo controlled, R=randomised, DB= double blind, MC=multicentre

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Treatment of Rheumatoid arthritis Efficacy study Mur *et al.* 2002	PC, R, DB Duration: 52 weeks, two phases: 24+28 weeks	Treatment: Phase one: Capsules of a commercial product (HE) (3 capsules/day, 20 mg UT extract/capsule)+ sulfasalazine or hydroxychloroquine Phase two: Capsules of a commercial product (3 capsules/day, 20 mg UT extract/capsule) Control: placebo in the first phase, in the second phase every patient received treatment *Per os* Duration of treatment: 52 week-two phases: 24+28 weeks 3 drop-out because of inefficacy of the drug or adverse events not attributed to the drug intake	40 patients (37 evaluable) Mean age: 53.1 vs. 54.9 4.8% vs. 21% male (HE vs. placebo) Treatment: 19 Control: 18 patients in phase one	Aged 20 years or more who fulfilled the American College of Rheumatology criteria for RA with Steinbrocker functional class II or III. Disease was considered active when 3 out of 4 criteria were fulfilled: ≥6 painful joints, ≥3 swollen joints, morning stiffness>30 mins, erythrocyte sedimentation rate>25 mm/h, or C-reactive protein >20 mg/L.	Phase one (vs. placebo) Painful joints reduction: 53.2 vs. 24.1% (p=0.001) Number of tender joints reduction: 50.65 vs. 24.1% (p=0.001) Ritchie index reduction: 49.1 vs. 12.5% (p=0.002) Duration of morning stiffness reduction: 47.8 vs. 21.7% (p=0.002) No significant differences in tender joints Phase two (vs. baseline or 24 weeks values in case of placebo) Number of tender joints Reduction: HE: 67.5% (p=0.001) Pl+HE: 57.1% (p=0.001) Number of swollen joints reduction: Pl+HE:44.6% (p=0.007) Ritchie index reduction: HE: 56.4% (p=0.001) Pl+HE: 57.1% (p=0.004) Duration of morning stiffness reduction (grade): HE: 34.8% (p=0.004)	For comparison of dependent variables at different times the nonparametric Wilcoxon and the Friedman test were used; the Mann-Whitney test was applied for comparison between the HE and the placebo group. S p value<0.05 was considered significant.	The TOA (tetraciylic alkaloid) free HE had a clinically relevant therapeutic potential in coadministrati on with conventional drugs and alone as well in the proposed indication.

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Treatment of Rheumatoid arthritis Efficacy study Castañeda *et al.* 1998	PC, R, DB, MC Duration: 6 months	Treatment: 2X3 capsule/day (commercial product) 150 mg *U. tomentosa* microspray/capsule Control: placebo *Per os* Duration of treatment: 6 months	70 patients (60 evaluable) Treatment: 29 patients Control: 31 patients (10 drop out, from which 2 because of adverse events not attributed to the drug intake)	Rheumatoid arthritis patients Inclusion criteria: Diagnosed with RA and who fulfilled the American College of Rheumatology criteria for RA, a necessary to start or change to NSAID, haven't used corticosteroids before 6 months of the study, if patients on low strength of anti-rheumatic medication the duration of it has to be more than a year, don't start new medication during study	Duration of morning stiffness reduction: 65.5 vs. +22.7% ($p<0.01$) Daytime pain reduction (scale): 48.3 vs. 14.3% ($p<0.001$) Night-time pain reduction (scale): 57.8 vs. 12.7% ($p<0.01$) Number of painful joints reduction: 70.59 vs. 9.4% ($p<0.001$) Number of swollen joints reduction: 65.3 vs. 2.4% ($p<0.001$) Sedimentation rate: 46.3 vs. 6.1% ($P<0.05$) Hematocrit: +5.4% vs. -10.6% ($p<0.05$)	Student t-test and Chi square for resulted values, coefficient of variation to determine the homogeneity	The product demonstrated significant effectiveness in every studied parameters, which overlap with the proposed indication in the monograph

Immunostimulant effect, antioxidant status

Uncaria tomentosa (UT)(side effect minimising and antioxidant status improving properties were investigated in a randomised interventional study, conducted on colorectal cancer (CRC) patients submitted to chemotherapy (Farias *et al.* 2012). Patients (n=43) undergoing adjuvant/palliative chemotherapy with FOLFOX-4 [5-Fluorouracil (1 g/m^2 on days 1+2)/leucovorin (200 mg/m^2 on days 1+2) + oxaliplatin (85 mg/m2 on day 1)] were split into two groups: the UT group (n=20) received chemotherapy plus **300 mg of *Uncaria tomentosa*** supply (commercial product**,** 3 tablets daily from day 3 to day 15) and the C group (n=23) received only FOLFOX4 served as a control. Each tablet contained **100 mg dry 70% ethanolic extract of the bark (not specified) of *Uncaria tomentosa*** with no TOA, and 2.57% POA content. Patients remained on study during 6 chemotherapies cycles, of 15 days each. One aim of the study was to evaluate neutropenia, thrombocytopenia, and anaemia. The haemograms were analysed each 15 days, and there were no significant differences in haematological parameters (e.g.: WBC, RBC, or platelet counts) between the groups for any of the cycles examined. The second aim was to evaluate the oxidative stress values (TBARS levels, protein carbonyl levels), activity of the antioxidant enzymes (catalase and SOD), DNA damage (comet assay) and the absolute count and ratio of CD4+ T cells and CD8+ T cells (used for the evaluation of the immune status of CRC patients). Between the groups, considering these values, there were no significant differences. The use of 300 mg of *Uncaria tomentosa* daily during 6 cycles of FOLFOX4 did not change the analysed parameters, and no toxic effects were observed. *Uncaria tomentosa* supplementation did not alter the occurrence of AEs, related to chemotherapy, neither caused adverse events. Treatment with UT did not alter liver function, defined as elevation of liver enzymes and kidney function.

Uncaria tomentosa side effect minimising property (leucopenia, neutropenia) was investigated by Araújo *et al.* (2012) in a randomised interventional study, conducted on patients with breast cancer (Invasive Ductal Carcinoma—Stage II) submitted to chemotherapy. Patients (n=40) undergoing a treatment regimen known as FAC (Fluorouracil, Doxorubicin, Cyclophosphamide), were divided into two groups: the UT (n=20) received chemotherapy plus **300 mg dry *Uncaria tomentosa* extract (70% ethanolic bark extract** in commercial product, 3 tablets per day) daily, from day 2 to day 21 and the Ca (n=20) group received only chemotherapy. Each tablet contained **100 mg dry 70% ethanolic extract of the bark (not specified) of *Uncaria tomentosa*** with no TOA, and 2.57% POA content. Healthy women in group C (n=20), with similar age of the patients were also investigated. Patients were part of the study during 6 chemotherapy cycles, of 21 days each. A greater reduction in the white blood cell (end of the treatment 3247/mm^3 (Ca) vs. 5469/mm^3 (UT), $p<0.05$) and the neutrophil counts (1083/mm^3 (Ca) vs. 4016/mm^3 (UT), respectively, $p<0.05$) were observed in the Ca group along the treatment, differently from the UT group, which remained closely the reference values, obtained in the control group. Monocytes number in patients with breast cancer (treated and not treated with UT) at 5-6 chemotherapy cycles was higher than control group, but in the UT group, this increase was stronger (817/mm^3 (UT) 500.9/mm^3 (Ca), $p<0.05$). During the chemotherapy treatment cycles, no significant difference was observed between groups with regards to CD4+ T cells, CD8+ T cells (absolute count and ratio) and IL-6 levels. Antioxidant defenses were analysed by the activity of Superoxide Dismutase (SOD) and Catalase (CAT) compared to treatment cycles zero and six, as well as between the UT and the Ca groups. There were no statistically significant differences among groups. Lipid peroxidation was estimated by the TBARS scale and the carbonylation of serum proteins, but there was no difference between groups (UT and Ca). The protective effect of chemotherapy to extract UT was evaluated by comet assay. In the sixth cycle (end of the treatment), it was observed a significant decrease in the comet assay index in the UT group, when compared to the Ca group ($p<0.05$).

Thirteen individuals with HIV-infection, who refused to receive other therapies, voluntarily took 20 mg per day of a **hydrochloric acid extract of** Uncariae tomentosae ***radix*** (containing 12 mg total

pentacyclic oxindole alkaloids per g and 300 mg sodium chloride per g as remnant from neutralisation; 10% yield from crude drug) (Keplinger *et al.* 1999). The test persons were 24–38 years-old adults (mean 33 years, 84% male). Blood tests were performed at the beginning and after 2.2–5.0 months of intake. Although the total leucocyte number remained unchanged within the whole collective, it was found that low values (<4000 per µl; 2 of 13) were raised and high values (>9000 per ml; 2 of13) were lowered. The relative and absolute lymphocyte count increased significantly in the 13 test persons 33.7 vs. 24% ($p<0.002$) compared to baseline. The four cases that were below normal (<20%) were raised above this level. However, no significant changes of T4/T8 cell ratios were observed.

Lamm *et al.* (2001) designed a placebo controlled, randomised intervention study (n=23). Healthy Caucasian male volunteers (n=11), aged 40-60 years old were given 350 mg of a commercially available filtered aqueous extract from the bark (not specified) of *Uncaria tomentosa* (AE), standardised to 8–10% CAEs (carboxy alkyl esthers) and almost free from oxindole alkaloids (≤0.05%),over a 60 days period twice a day. On day 30 all study subjects were vaccinated with 23 valent pneumococcal vaccine (serotypes 1, 3, 4, 6, 8, 9N, 12, 14, 19F, 23F, 51 and 56), on day 180 there was a post vaccination follow-up. There was a statistically significant elevation in the lymphocyte/neutrophil ratios in peripheral blood at day 60 in AE treated group (0.38 before, 0.55 after ($p=0.05$), but numbers of neutrophils decreased with 841db/mm^3 ($p=0.036$) in the AE group after 60 days of treatment. The total white cell blood counts (WBC) (700/mm3 decrease, $p=0.089$) and percentages of neutrophils (5.8% decrease $p=0.089$) and lymphocytes (6.1% increase, $p=0.064$) tended to be statistically altered from AE treatment. Furthermore immunisation levels for the 12 serotype antibody titers (% of antibody titers) was significantly greater in the placebo group compared to AE group, 72.8 and 52.3%, respectively. Presumably this outcome is because of a bias, caused by the lack of randomisation between the two groups for natural immunisation to pneumococcal infection. The controls had 44.8% natural immunisation levels against pneumococci compared to the AE group, which had only 31.3% before the vaccination. On the other hand, there was no loss of pneumococcal immunity in the AE group, but a reduced decay in the 12 serotype antibody titer responses to pneumococcal vaccination at day 180, whereas the controls experienced a highly significant decrease in pneumococcal immunity. There were no toxic side effects observed as judged by medical examination, clinical chemistry and blood cell analysis. There were also no documented side effects attributed to AE supplementation or pneumococcal vaccination during the trial evaluation period of 180 days. The mineral status did indicate a statistically significant but clinically insignificant decrease in serum sodium and a comparable increase in iron.

In a human volunteer study, **a commercially available filtered aqueous extract from the bark (not specified) of *Uncaria tomentosa*** (AE), standardised to 8–10% CAEs (carboxy alkyl esthers) and almost free from oxindole alkaloids (≤0.05%), was given daily at 5 mg/kg for 6 consecutive weeks to four healthy adult (mean age: 46.1 years) males in the form of 350 mg tablets (Sheng *et al.* 2000a). Total blood cell counts were used to monitor the efficacy and toxicity. There were no toxic side effects judged by haematological analysis, bodyweight loss, work attendance and symptoms (diarrhoea/constipation, headache, nausea/vomiting, rash/edema and pain). WBC showed a significantly increased level ($6.60X10^9$/l before vs. $7.18X10^9$/l after).

Table 6: Clinical studies on humans, on immunostimulant activity

Abbreviations: PC=placebo controlled, R=randomised, O=Open

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Immunological changes post-vaccination Intervention study Lamm *et al.* 2001	PC, R Duration: 180 days, at day 30 vaccination (23 valent pneumococcal vaccine) at day 180 follow up	Treatment: commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE) 350 mg two times daily Control: placebo Per os Duration of treatment: first 60 days	23 patients 100% male Mean age: 48 Treatment: 11 patients Control: 12 patients	Healthy Caucasian male patients Inclusion criteria: no previous pneumococcal vaccination, no active chronic disease, and no concurrent medication	12 pneumococcal anti-body titers (% and concentration) at day 60: 52.5 vs. 72.8% ($p<0.05$) (C-AE vs. placebo) 12 pneumococcal anti-body titers (% decay): sig. lower in AE group Lymphocyte/neutrophil ratios: 0.38 before, 0.55 after AE treatment ($p=0.05$) Neutrophil leucocytes Day 60: 841/mm3 decrease in AE group ($p=0.036$) WBC count: 700/mm3 decrease in AE group ($p=0.089$) % of neutrophil leukocytes: 5.8% decrease in AE group ($p=0.089$): % of lymphocytes: 6.1% increase in AE group ($p=0.064$)	Comparison of means between control and supplement groups: two-tailed t-test. Comparison before and after supplement for the supplement group: paired t-test. Comparison of the percentage of the immunised levels of pneumococcal antibody titers: chi square test. Level of significance: 0.05.	Study has weak evidence to support the immunostimulant activity of AE after pneumococcal vaccination. Several parameters showed just trends.
Adjuvant treatment for colorectal cancer (side effect, neutropenia minimising effect). Antioxidant effect Interventional study Farias *et al.* 2012	R Duration: not specified	Treatment: UT group: commercial product, 3 tablets daily, 100 mg 70% ethanolic extract/tablet+ FOLFOX4 Control: FOLFOX4 Per os Duration: 6	43 patients Mean age: 62.7 vs. 60.9 50% vs. 30.4% male (UT+FOLFOX4 vs. FOLFOX4) Treatment: 20 patients Control: 23 patients	colorectal cancer (CRC) patients under chemotherapy (5-Fluorouracil/leucovorin + oxaliplatin-FOLFOX4) Inclusion criteria: Subjects had undergone complete resection of their colorectal cancer, which was of histologically scored as stage IIB, III, or IV, and they were	No significant differences: After every cycle (15 days of interval) Hematological parameters TBARS and protein carbonyl levels, catalase and SOD activity, DNA damage, absolute count and ratio of CD4+ T cells and CD8+ T cells	The data were evaluated using analysis of variance (ANOVA) and t-test. When the variances were not homogenous and ANOVA was not appropriate (Bartlett's P value < 0.05), Wilcoxon two-sample test	*U. tomentosa* at dose 300 mg dry extract daily is not effective in reducing the most prevalent adverse events due to treatment with 5FU/Leucovorin and oxaliplatin in patients with advanced CRC.

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
		chemotherapy cycles 15 days each *U. tomentosa* extract: from day 3 to day 15		going to begin adjuvant/palliative chemotherapy with FOLFOX4 at the Hospital Universitaʹrio de Santa Maria, Brazil.		was used to evaluate the data. P < 0.05 was considered statistically significant	
Adjuvant treatment for breast cancer (side effect, leucopoenia, neutropenia minimising effect) Antioxidant effect Interventional study Araújo *et al.* 2012	R Duration: not specified	Treatment: UT group: commercial product, 3 tablets daily, 100 mg *U. tomentosa* 70% ethanolic extract/tablet+ FAC Ca group: FAC Cl group: no medication Per os Duration of treatment: 6 chemotherapy cycles 21 days each *U. tomentosa* extract: from day 2 to day 21	60 patients Mean age: 54.4 vs. 55 vs. 56.5 (UT vs. Ca vs. Cl) Treatment: 20/20 patients (UT/Ca) Control: 20 healthy patients	Breast cancer patients under chemotherapy FAC (Fluorouracil, Doxorubicin, Cyclophosphamide) Inclusion criteria: Subjects had undergone complete breast cancer resection, which was his-tologically diagnosed as Invasive Ductal Carcinoma—Stage II, and they were going to begin adjuvant chemotherapy with Doxorubicin-based scheme for six cycles, at the Santa Maria University Hospital, Brazil. Control group: healthy women, with similar age of the patients and that did not receive any medication in the last 30 days or have chronic disease.	WBC, end of 6th cycle, Ca vs. UT: 3247/mm3 vs. 5469/mm3 (p<0.05) Neutrophils, end of 6th cycle, Ca vs. UT: 1083/mm3 vs. 4016/mm3 (p<0.05) Monocytes, end of 6th cycle, Ca vs. UT: 500.9/mm3 vs. 817/mm3 (p<0.05) No significant difference: TBARS, SOD, CAT activity, absolute count and ratio of CD4+ T cells and CD8+ T cells Protective effect on DNA-damage (comet assay) end of the 6th cycle: significant decrease (UT) in the index test (p<0.05)	For the statistical analysis Student's t-test was used. P < 0.05 was considered to represent a significant difference in all tests, statistical power 90%.	Treatment using a daily dose of 300 mg dry *U. tomentosa* extract was effective in reducing the main chemotherapy side effect, neutropenia at similar posology as proposed in the monograph. Antioxidant effect wasn`t the part of the mechanism of action.

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Immunostimulant activity in healthy patients Observational study Sheng *et al.* 2000a	Open study Duration: 9 weeks (3+6 weeks)	Treatment: 350 mg of a commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE) daily Per os Duration of treatment: 6 weeks	4 patients Mean age: 46.2 (32-58)	Healthy males	WBC levels after 6 weeks: 7.18X109/l before vs. 6.60 X109/l after ($p<0.05$)	Two-tailed t-test was used to compare the before and after supplementation values. Level of significance: 0.05	Although the result supports the immunostimulant activity of *U. tomentosa*, the population of the study is extremely small, so it considered as weak evidence.
Immunostimulant activity in HIV patients Observational study Keplinger *et al.* 1999	Open study Duration: 2.2-5 months	Treatment: 20 mg *U. tomentosa* root daily (containing 12 mg total pentacyclic oxindole alkaloids per g Per os Duration of treatment: 2.2-5 months	13 patients Mean age: 33 (24-38 years) 84% male	Adults with HIV infection, inclusion criteria not specified	Total leucocyte number remained unchanged, low values (<4000 per µl; 2 of 13) were raised and high values (>9000 per ml; 2 of13) were lowered Increase of lymphocytes: 33.7 before vs. 24% after ($p<0.002$) No significant changes of T4/T8 cell ratios	T-test for paired samples	Weak evidence of immunostimulant activity, poorly planned, even the duration of treatment is heterogenic, conclusions hard to draw

Herpes simplex labialis

The effect of an *Uncaria tomentosa* bark (not specified) extract containing cream (UC, gel-cream 50mg extract/g) was tested against herpes labialis lesions in a reference drug controlled, randomised double blinded clinical trial by Caldas *et al.* (2010). The extract was standardised to a content of 5% mitraphyllin. From 74 healthy volunteers with positive history of recurrent herpes, 54 episodes of *herpes labialis* lesions developed in 31 volunteers during the study period. Hence, 27 patients received the reference drug (Acyclovir containing cream) while 27 applied UC at least four times a day. The primary outcomes referred to: I. time for complete resolution (therapy length in days); II. time to drying or to start crust formation (in days); III. clinical course and intensity of signs and symptoms (pruritus, tension, pain, swelling, erosion, diameter of largest lesion) at scheduled evaluation visits. Median episode length was 8.4 days and 8.1 days, the inflammatory period was 6.7 days and 7 days and the last stage (crust formation) was 2.7 and 3 days, for UC and Acyclovir, respectively. There was no statistically significant difference either in total episode length or in inflammatory or crust formation time course. However, symptom scores registered by patients reflecting their intensity, UC group showed, during clinical course, significantly lower scores on the first two days of treatment ($p<0.005$; $t=0.028$), in terms of intensity of inflammatory signs (swelling, erythema) and symptoms (pain), its efficacy was significantly superior to acyclovir. From third day on, there was no statistically significant difference. Regarding the severity of inflammatory reaction, the clinical efficacy of *Uncaria tomentosa* was significantly better than acyclovir. Both drugs were, overall, well tolerated, and no adverse events occurred.

Table 7: Clinical studies on humans, on *Herpes labialis* infection

Abbreviations: C= controlled, R=randomised, DB= double blind

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Treatment of *Herpes labialis* Comparative study of *U. tomentosa* cream (UC) and acyclovir cream Caldas *et al.* 2010	C, R, DB Duration: not specified	Treatment: *U. tomentosa* cream (UC) Control: acyclovir cream Both at least 4 times daily Topical application Duration of treatment: until resolution	74 patients with 54 episodes Mean age: 28.1 vs. 30.6 17.4% vs. 12.5% male (episodes treated with UC vs. Treatment: 27 episodes Control: 27 episodes	Healthy adults with positive history of recurrent herpes labialis simplex	UC vs. acyclovir Length of therapy (days): Median: 8.4 vs. 8.1 Time to drying/crust formation (days): Median 2.7 vs. 3 Inflammatory period (days): Median 6.7 vs. 7 Intensity of signs and symptoms: significantly lower scores on the first two days of treatment ($p < 0.005$; $t = 0.028$ Satisfaction with treatment: 100%	Not specified	Both drugs were effective and safe for herpes labialis treatment. The only difference between them was in the intensity of inflammatory signs and symptoms, UC was significantly superior to reference drug.

Alzheimer's disease

A proprietary extract of the root or the inner bark of *Uncaria tomentosa* (PE, no further details on the extract) was tested in a pilot study for treatment of Alzheimer's disease. PE at the dose of 350 mg *per os*, three times a day, for one year failed to slow the rate of clinical decline or the rate of brain atrophy in patients with Alzheimer's. The treatment period was one year. Clinical outcome measures included the mini-mental state examination (MMSD) and the Alzheimer's disease assessment scale (ADAS-cog). Additional outcome measures included rates of brain atrophy, completed at baseline and at 12 months, and CSF levels of β-amyloid 1-42, τ, and F2 isoprostanes taken at the baseline, 3 months and 12 months. The study population at baseline consisted of 26 man and 14 women, with a mean age of 71±7 years, mean MMSE=19.5±5; mean ADAS=24±10; mean ADL (activities of daily living) =31±11; IADL (mean instrumental activities of daily living) =6±3. All 40 subjects completed the first 3 months of the trial and β-amyloid levels in CSF samples were determined in 39 of 40 patients. There was no significant difference between groups. All 20 subjects on placebo completed all 12 months of the study. Four subjects on active treatment dropped out between 3 and 12 months. None of the clinical measurements distinguished PE-treated subjects from placebo. All of 36 the subjects completing the study had another brain MRI at 12 months with measurement of hippocampal volume, total brain volume, temporal lobe volume, and total ventricular volume, and annual rates of volume change in each of these regions was calculated. 33 of the 36 subjects completing the study had CSF collected at 12 months. There were no significant differences in change in brain volumes and in CSF β-amyloid levels between groups (Quinn *et al.* 2004).

Table 8: Clinical studies on humans, on Alzheimer's disease

Abbreviations: PC= placebo-controlled

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Treatment of Alzheimer's disease Quinn *et al.* 2004	Pilot study, PC Duration: 1 year	Treatment: A proprietary extract of the root or the inner bark of *U. tomentosa* (PE), three times daily (3X350 mg) *Per os* Duration of treatment: 1 year	40 patients Mean age=71±7 years 65% male Treatment: 20 patients Control: 20 patients 4 drop outs after 3 months (death, adverse events, noncompliance, withdrawal of consent) 3 subjects missing CSF β-amyloid levels at 12th month	Patients with Alzheimer's disease	None of the clinical measurements (mini-mental state examination, Alzheimer disease assessment scale) distinguished PE-treated subjects from placebo 3rd month (40 subjects): There were no significant differences in change in CSF β-amyloid levels between groups 12th month (36 and 33 subjects): There were no significant differences in change in brain volumes and in CSF β-amyloid levels between groups.	Not discussed	PE was ineffective to slow the rate of clinical decline or the rate of brain atrophy in the sample of subjects with Alzheimer's disease at the administered dose. No effect of PTI-00703 upon CSF â-amyloid level was the apparent.

Antimutagenic activity

Two healthy donors, one smoker and one non-smoker were requested to drink daily for 15 days a decoction of *Uncaria tomentosa* (about 6.5 g bark/day). The two donors were males, both 35 years old, one smoker for over 15 years (20 cigarettes a day). The decoction was obtained by boiling in water the dried bark for 3 hours until the volume decrease to about one third, according to the method used in traditional medicine. Urine from the two subjects was collected before, during (day 8) and after the last treatment. The non-smoker's urine did not show any mutagenic activity (AMES test). The smoker's urine had mutagenic activity before treatment, which showed a dramatic decrease of mutagenic potential at the end of the treatment, persisting until after the end of the treatment (Rizzi *et al.* 1993).

In a double-blind, placebo controlled randomised study the effects of a freeze-dried aqueous extract of *Uncaria tomentosa* bark was assessed on the mutagenic activity of urine. For an average of 32 days 24 volunteers of whom half were smokers received placebo or the bark extract in two doses (90 mg or 270 mg/day). The urine of the non-smokers was free from mutagenic activity (AMES test) upon entry to the study. On days 17 and 32 of the treatment period, they found, that all those taking the bark extract showed no mutagenic activity in their urine samples, whereas no change in mutagenic activity showed up in urine samples from those on placebo. In the smokers, the dosage of bark extract consumed showed a linear and significant correlation with the decrease of assayed mutagenic activity (Leon *et al.* 1996).

Table 9: Clinical studies on humans, on antimutagenic activity

Abbreviations: C= controlled, R=randomised, DB= double blind

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Efficacy study on antimutagenic activity in smokers Interventional study Rizzi *et al.* 1993	Open study Duration: 15 days	Treatment+control: about 6.5 g *U. tomentosa* bark/day as decoction *Per os* Duration of treatment: 15 days	2 patients both males, 35 years old Treatment: 1 patient smoker Control: 1 patient non-smoker	A smoker (smokes for over 15 years about 20 cigarettes a day) and a non-smoker adult. Neither of them had been radiologically or pharmacologically treated for over 6 months before the test, and both were free from viral diseases.	Ames test for detecting antimutagenic activity in urine, 15th day : “dramatic decrease of mutagenic potential” , non-smoker didn’t have mutagenic activity	Not specified	An apparent antimutagen activity was recorded, but the sample size comprised only 2 subjects, thus this data is weak support of the efficacy.
Efficacy study on antimutagenic activity in smokers Leon *et al.* 1996	C, R, DB Duration: 32 days	Treatment: freeze-dried aqueous extract of *U. tomentosa* bark, 90 mg or 270 mg/day *Per os* Duration of treatment: 32 days	24 patients	12 non-smokers, 12 smokers	Ames test for detecting antimutagenic activity in urine: the urine of the non-smokers was free from mutagenic activity upon entry to the study. 17th and 32nd day: urine of the UT treated patients showed no mutagenic activity; urine of the placebo treated subjects had no change in mutagenic activity. In the smokers, the dosage of bark extract consumed showed a linear and significant correlation with the decrease of assayed mutagenic activity.		An apparent antimutagen activity was recorded, but the sample size is small. Nevertheless, it had the same outcome as in the study of Rizzi *et al.* (1993).

4.3. Clinical studies in special populations (e.g. elderly and children)

No relevant data available

4.4. Overall conclusions on clinical pharmacology and efficacy

The assessment report presents 12 clinical studies, investigating the effect of *Uncaria tomentosa* on immunstimulation (colorectal, breast cancer, HIV patients and healthy adults), Alzheimer's disease, rheumatoid arthritis, herpes simplex labialis, antioxidant status (colorectal and breast cancer patients) and mutagenicity (healthy adults). Overall seven studies were randomised, four double-blind and six placebo-controlled. Almost half of the trials were open studies, where the lack of true control group, blinding and randomisation limits the usefulness of these trials.

The posology regarding the dosage and formulation and study population are quite heterogeneous. Moreover, four out of the 12 studies had a population less than 20. In case of some trials the concomitant medication prevents the objective evaluation.

The immunostimulant effect of *Uncaria tomentosa* is rather controversial in the five presented studies. The two studies, regarding rheumatoid arthritis showed significant treatment effects for the alleviation of symptoms. Considering the small sample size more trials are needed in order to allow a firm conclusion to be drawn on the use of *Uncaria* extract in the treatment of rheumatoid arthritis.

Based on the one performed study on treatment of *herpes simplex labialis* in adults, *Uncaria tomentosa* seemed to act mostly as an anti-inflammatory agent, not as an antiviral one.

Three studies investigated the DNA-repair, but because of the small sample size clear conclusion cannot be drown. The antioxidant activity of *Uncaria tomentosa* was not proved in two studies.

A proprietary extract of the root or the inner bark of *Uncaria tomentosa* was ineffective in Alzheimer's disease.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

The safety of clinical trials was assessed with respect to the adverse events and the results of laboratory test.

Table 10: Clinical safety data from clinical trials on antimutagenic activity

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis
DNA damage and repair Efficacy study Sheng *et al.* 2001	PC, R Duration: 11 weeks (3+8)	Treatment: 350/250 mg/day a commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE) Control: placebo *Per os* Duration of treatment: 8 weeks	12 patients 50% male in every group Mean age: 43.8 vs. 43.3 vs. 44 (350 mg/day vs. 250 mg/day AE vs. (placebo) Treatment: 4/4 patients 350/250 mg/day Control: 4 patients	healthy patients	No adverse effects, in terms of symptoms and signs, whole blood hematological analysis, or blood chemistry analysis have been found.	No adverse effects.
Efficacy study on antimutagenic activity in smokers Interventional study Rizzi *et al.* 1993	Open study Duration: 15 days	Treatment+control: about 6.5 g *U. tomentosa* bark/day as decoction *Per os* Duration of treatment:15 days	2 patients both males, 35 years old Treatment: 1 patient smoker Control: 1 patient non-smoker	A smoker and a nonsmoker adult. Smoker smokes for over 15 years about 20 cigarettes a day. Neither of them had been pharmacologically or radiologically treated for over 6 months before the test, and both were free from viral diseases.	Not discussed	Safety of the preparation was not discussed.
Efficacy study on antimutagenic activity in smokers Leon *et al.* 1996	C, R, DB Duration: 32 days	Treatment: freeze-dried aqueous extract of *U. tomentosa* bark, 90 mg or 270 mg/day *Per os* Duration of treatment: 32 days	24 patients	12 non-smokers, 12 smokers	Not discussed	Safety of the preparation was not discussed.

Table 11: Clinical safety data from clinical trials on rheumatoid arthritis

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis
Treatment of Rheumatoid arthritis Efficacy study Mur *et al.* 2002	PC, R, DB Duration: 52 weeks, two phases: 24+28 weeks	Treatment: Phase one: Capsules of a commercial product (HE) (3 capsules/day, 20 mg UT extract/capsule)+ sulfasalazine or hydroxychloroquine Phase two: Capsules of a commercial product (3 capsules/day, 20 mg UT extract/capsule) Control: placebo in the first phase, in the second phase every patient received treatment *Per os* Duration of treatment: 52 week- two phases: 24+28 weeks 3 drop-out because of inefficacy of the drug or adverse events not attributed to the drug intake	40 patients (37 evaluable) Mean age: 53.1 vs. 54.9 4.8% vs. 21% male (HE vs. placebo) Treatment: 19 Control: 18 patients in phase one	Aged 20 years or more who fulfilled the American College of Rheumatology criteria for RA with Steinbrocker functional class II or III. Disease was considered active when 3 out of 4 criteria were fulfilled: ≥6 painful joints, ≥3 swollen joints, morning stiffness>30 mins, erythrocyte sedimentation rate>25 mm/h, or C-reactive protein >20 mg/L.	During the first phase, adverse events occurred in 12 patients of each group (e.g.: Dyspepsia, respiratory infection, dermatitis, pruritus, gastritis, headache). In the second phase, 7 other side effects were seen; none could be clearly attributed to the drug intake. No major side effects were seen in the active and the placebo group.	HE was used in combination with sulfasalazine or hydroxychloroquine. Both the number and quality of side effects of the HE were comparable to placebo.
Treatment of Rheumatoid arthritis Efficacy study Castañeda *et al.* 1998	PC, R, DB, MC Duration: 6 months	Treatment: 2X3 capsule/day (commercial product) 150 mg *U. tomentosa* microspray/capsule Control: placebo *Per os* Duration of treatment: 6 months	70 patients (60 evaluable) Treatment: 29 patients Control: 31 patients (10 drop out, from which 2 because of adverse events not attributed to the drug intake)	Rheumatoid arthritis patients Inclusion criteria: Diagnosed with RA and who fulfilled the American College of Rheumatology criteria for RA, a necessary to start or change to NSAID, haven`t used corticosteroids before 6 months of the study, if patients on low strength of antirheumatic medication the duration of it has to be more than a year, don`t start new medication during study	25.8% of the treat-ment group and 13.8% of the control group had adverse reactions, most frequently gastric disturbances. Other adverse reactions were: nausea, diarrhoea, bitter mouth, meteorism and constipation. There was no statistically significant difference between the two groups in regard to the adverse reactions (p=0.245).	There was no statistically significant difference between the two groups in regard to the adverse reactions.

Table 12: Clinical safety data from clinical trials on immunstimulant activity

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis
Immunological changes post-vaccination Intervention study Lamm *et al.* 2001	PC, R Duration: 180 days, at day 30 vaccination (23 valent pneumococcal vaccine) at day 180 follow up	Treatment: commercially available filtered aqueous extract from the bark of *U. tomentosa* (AE), 350 mg two times daily Control: placebo *Per os* Duration of treatment: first 60 days	23 patients 100% male Mean age: 48 Treatment: 11 patients Control: 12 patients	Healthy Caucasian male patients Inclusion criteria: no previous pneumococcal vaccination, no active chronic disease, and no concurrent medication	There were no toxic side effects observed as judged by medical examination, clinical chemistry and blood cell analysis. There were also no documented side effects attributed to AE supplementation or pneumococcal vaccination during the trial evaluation period of 180 days. The mineral status did indicate a statistically significant but clinically insignificant decrease in serum sodium and a comparable increase in iron.	There were no clinically relavant findings retated to the safety of the preparation.
Adjuvant treatment for colorectal cancer (side effect, neutropenia minimising effect). Antioxidant effect Interventional study Farias *et al.* 2012	R Duration: not specified	Treatment: UT group: commercial product, 3 tablets daily, 100 mg 70% ethanolic extract/tablet+ FOLFOX4 Control: FOLFOX4 *Per os* Duration: 6 chemotherapy cycles 15 days each *U. tomentosa* extract: from day 3 to day 15	43 patients Mean age: 62.7 vs. 60.9 50% vs. 30.4% male (UT+FOLFOX4 vs. FOLFOX4) Treatment: 20 patients Control: 23 patients	colorectal cancer (CRC) patients under chemotherapy (5-Fluorouracil/leucovorin + oxaliplatin-FOLFOX4) Inclusion criteria: Subjects had under-gone complete resection of their colorectal cancer, which was of histologi-cally scored as stage IIB, III, or IV, and they were going to begin adjuvant/palliative chemotherapy with FOLFOX4.	Adverse events related to antineoplastic drugs (oxaliplatin and 5FU) are well known and are similar to those observed in our study e.g. Leucopenia, diarrhoea, hyperglycemia, weight loss). No toxic effects related to UT were observed.	No toxic effects related to UT were observed.

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis
Adjuvant treatment for breast cancer (side effect, leukopenia, neutropenia minimising effect) Antioxidant effect Interventional study Araújo *et al.* 2010	R Duration: not specified	Treatment: UT group: commercial product, 3 tablets daily, 100 mg *U. tomentosa* 70% ethanolic extract/tablet+ FAC Ca group: FAC Cl group: no medication *Per os* Duration of treatment: 6 chemotherapy cycles 21 days each *U. tomentosa* extract: from day 2 to day 21	60 patients Mean age: 54.4 vs. 55 vs. 56.5 (UT vs. Ca vs. Cl) Treatment: 20/20 patients (UT/Ca) 20 patients	Breast cancer patients under chemotherapy FAC (Fluorouracil, Doxorubicin, Cyclophosphamide) Inclusion criteria: Subjects had undergone complete breast cancer resection, which was his- tologically diagnosed as Invasive Ductal Carcinoma—Stage II, and they were going to begin adjuvant chemotherapy with Doxorubicin-based scheme for six cycles, at the Santa Maria University Hospital, Brazil. Control group: healthy women, with similar age of the patients and that did not receive any medication in the last 30 days or have chronic disease.	Not discussed	Safety of the preparation was not discussed.
Immunostimulant activity in healthy patients Observational study Sheng *et al.* 2000a	Open study Duration: 9 weeks (3+6 weeks)	Treatment: 350 mg of a commercially avail- able filtered aqueous extract from the bark of *U. tomentosa* (AE) daily *Per os* Duration of treatment: 6 weeks	4 patients Mean age: 46.2 (32-58)	Healthy males	There were no toxic side effects judged by hematological analysis, body-weight loss, work attendance and symptoms (diarrhoea/constipation, headache, nausea/vomiting, rash/edema and pain)	There were no toxic side effects.

Immunostimulant activity in HIV patients Observational study Keplinger *et al.* 1999	Open study Duration: 2.2-5 months	Treatment: 20 mg *U. tomentosa* root daily (containing 12 mg total pentacyclic oxindole alkaloids per g *Per os* Duration of treatment: 2.2-5 months	13 patients Mean age: 33 (24-38 yrs) 84% male	Adults with HIV infection, inclusion criteria not specified	Not discussed	Safety of the preparation was not discussed.

Table 13: Clinical safety data from clinical trials on herpex simplex labialis

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis
Treatment of *Herpes labialis* Comparative study of *U. tomentosa* cream (UC) and acyclovir cream Caldas *et al.* 2010	C, R, DB Duration: not specified	Treatment: *U. tomentosa* cream (UC) Control: acyclovir cream Both at least 4 times daily Topical application Duration of treatment: until resolution	74 patients with 54 episodes Mean age: 28.1 vs. 30.6 17.4% vs. 12.5% male (episodes treated with UC vs. acyclovir) Treatment: 27 episodes: Control: 27 episodes	Healthy adults with positive history of recurrent herpes labialis simplex	No adverse reactions were reported	No adverse reactions were reported.

Table 14: Clinical safety data from clinical trials on Alzheimer's disease

Type	Study	Test Product(s)	Number of Subjects	Type of subjects	Outcomes	Statistical analysis
Treatment of Alzheimer's disease Quinn *et al.* 2004	Pilot study, PC Duration: 1 year	Treatment: A proprietary extract of the root or the inner bark of *U. tomentosa* (PE), three times daily (3X350 mg) *Per os* Duration of treatment: 1 year	40 patients Mean age=71±7 years 65% male Treatment: 20 patients Control: 20 patients 4 drop outs after 3 months (death, AE, noncompliance, withdrawal of consent) 3 subjects missing CSF β-amyloid levels at 12th month	Patients with Alzheimer's disease	1 adverse event during 1 year	The preparation can be considered safe based on the reported adverse reaction.

5.2. Patient exposure

The clinical trials referred in this assessment report (4.1) were conducted on approximately 370 adult patients. Among the human studies two were conducted on 100 rheumatoid arthritis patients, five on 65 healthy adults, one on 13 HIV positive patients, one on 43 colorectal cancer patients (plus 20 healthy controls), one on 40 breast cancer patients, one on 31 *herpes labialis* patients, one on 40 Alzheimer‘s disease patients and one on 14 adults from which 78.6% used prescription medications for different diseases, such as cardiac disease, hypertension, diabetes, hysterectomy, thyroid disease, allergy, arthritis, and polio. No information is available from marketed products.

5.3. Adverse events, serious adverse events and deaths

During the studies in section 4.4 there were either no adverse reactions observed as judged by medical examination and blood chemistry analysis, or none could be clearly attributed to the drug intake, or there was no statistically significant difference between the experienced adverse effects between the placebo and the treatment group.

Case report 1 described reversible worsening of motor signs in a patient with Parkinson disease after oral intake of self-prepared hot extracts of the bark of *Uncaria tomentosa*, 3 cups daily for 3 weeks. The motor symptoms of the 38-year-old man have been well controlled, taking small frequent doses of levodopa and pergolide. He had some mild-to-moderate peak-of-dose dyskinesia, wearing-off phenomena, and some ‘‘sudden off’’ episodes. A few days after the first day of intake of the hot *Uncaria tomentosa* extract, tremor and hypokinesia markedly increased, being in off states most part of the days. Time and doses of levodopa and pergolide remained unchanged. After 3 weeks with the same poor response the patient stopped the consumption, but remained markedly hypokinetic for one more week. Then, and without any change in levodopa and pergolide daily dosing, motor symptoms progressively improved to reach the basal state previous to the intake of the extract (Cosentino and Torres, 2008)

Assessor's comment: Although *t*he symptoms improved after discontinuation of the use of the extract of the bark of *Uncaria tomentosa* the exact way of the preparation (hot?) and posology of the extract (what quantity of the herbal substance was contained in one cup) is missing. A conclusion cannot be drawn.

Case report 2: A 35-year-old woman with systematic lupus erythematosus was treated intermittently with immunosuppressive therapy for active episodes of lupus and regularly with prednisolone 12.5 mg/day, atenolol 100 mg/day, metolazone 5 mg/day, furosemide 40 mg/day and nifedipine 120 mg/day. After taking 1 capsules four times daily of Uncariae tomentosae herbal medication the patient was diagnosed with acute allergic interstitial nephritis with 3.6 mg/dl creatinine, pyuria, and red and white blood cell casts in the urinary sediment. 1 month after discontinuation of the herbal preparation, creatinene concentration was 2.7 mg/dl with no evidence of pyuria or white blood cells casts in the urine sediment. The patients, in all likelihood, experienced a “type B” or idiosyncratic adverse reaction to the herbal remedy. The exact ingredients and dosages of the herbal remedy were not available from the package insert (Hilepo *et al.* 1997).

Case report 3: A 59-year-old woman with mantle-cell lymphoma was under observation without therapy. There was no evidence of hepatic involvement. Her medications were shark cartilage (three capsules twice daily), bee pollen, beta-carotene, Echinacea, garlic, selenium, vitamin C and vitamin E. At a routine monitoring visit, her AST concentration was 309 U/l (compared with 27 U/l 3 months earlier; normal <9 U/L) and her ALT was 572 U/l (normal< 29 U/L). Additional history revealed the initiation within the prior month of therapy with una de gato (not specified). After withdrawing this

supplement, but continuing the other medications, 60 days later, her AST concentration had normalised (Gertz *et al.* 2001).

Assessor's comment: No details are available in the article about the preparation.

U.S. Department of Health and Human Services and National Centre for Complementary and Alternative Medicine states that few side effects have been reported for cat's claw when it is taken at recommended dosages. Though rare, side effects may include headaches, dizziness, and vomiting (NCCIH 2012).

Adverse effects relating to registered products:

No adverse effects were reported in the Spanish market overview.
Hungarian package leaflets of products containing Cat's claw mention the followings under the point of undesirable effects: gastrointestinal complaints such as heartburn, diarrhoea, constipation, flatulence may occur. The frequency is not known.

5.4. Laboratory findings

The clinical trial carried out by Farias *et al.* (2011) mentioned, that toxicity of the *Uncaria tomentosa* extract was also evaluated using liver, kidney, metabolic, and constitutive parameters. Treatment with this extract did not alter liver function, defined as elevation of liver enzymes (ALT, AST, γGT), and bilirubin levels, and kidney function is evaluated by dosage of urea, metabolic parameters (albumin levels and glycaemia), and constitutive parameters (weight loss).

Sheng *et al.* (2000a) reported that under the treatment of **a commercially available aqueous extract from the bark of *Uncaria tomentosa* (350 mg daily)**, there were no toxic side effects observed, when judged by haematological analysis, bodyweight loss, work attendance and symptoms. The parameters of the haematological analysis were not discussed.

Sheng *et al.* (2001) observed no statistically significant differences among the groups (two doses of **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE), placebo) in white blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelet (PLT), and numbers/ percentages of monocytes (MONO), eosinophils (EOS), basophils (BASO) and lymphocytes. There were also no statistically significant differences in plasma total protein, albumin, glucose protein, and iron, sodium, potassium, calcium, magnesium, triglycerides and cholesterol concentration among the groups of control, AE 250 mg/day and AE 350 mg/day.

Lamm *et al.* (2001) reported no statistically significant differences between the **a commercially available filtered aqueous extract from the bark of *Uncaria tomentosa*** (AE) supplemented group (350 mg two times daily) and controls for the serum chemical markers estimating liver (total bilirubin, direct bilirubin, alkaline phosphatase, γGT, SGOT, or AST, SGPT, ALT) and kidney function (blood urea nitrogen, creatine, their ratio, uric acid) or nutrient status (triglycerides, cholesterol, HDL, LDL, cholesterol/HDL ratio, LD, or LDH, iron, calcium, sodium, potassium, miscellaneous indicators including glucose, total protein, albumin, globulin). The mineral status did indicate a statistically significant but clinically insignificant decrease in serum sodium and a comparable increase in iron while the rest of the minerals measured remained unchanged (0.73% reduction $p=0.045$, and 38.14% increase $p=0.021$, respectively). Most of the blood cell parameters were unaffected by AE supplementation including RBC, HGB, HCT, MCV, MCH, mean corpuscular haemoglobin concentration, RBC distribution width, PLT count, volume, and numbers/percentages of MONO, EOS, BASO and lymphocyte subset analyses (i.e. absolute numbers and percentages of CD3, helper, suppressor T-lymphocytes.

Castañeda *et al.* (1998) reported, that laboratory parameters had significant changes, haematocrit elevated in the treatment group by 5.4% but reduced in the placebo by 10.76% ($p<0.05$). Treatment with *Uncaria tomentosa* (2X3 capsule/day of a commercial product, containing 150 mg *Uncaria tomentosa* microspray/capsule) did not alter glucose and creatinine levels, liver enzymes (AST, ALP, ALT) or urine sedimentation.

5.5. Safety in special populations and situations

To date, neither safety studies including women who are pregnant or breastfeeding, nor individuals with hepatic or renal disease have been performed.

No information is available on overdose, drug abuse and withdrawal.

5.5.1. Use in children and adolescents

No data available

5.5.2. Contraindications

No data available

5.5.3. Special Warnings and precautions for use

No data available

5.5.4. Drug interactions and other forms of interaction

Case report 4: A 45-year-old woman, HIV positive, with cirrhosis associated to hepatitis C infection, was referred to the hospital for clinical evaluation and, ultimately, for liver transplantation. Three months later, antiretroviral treatment was changed to abacavir (600 mg qd), lamivudine (300 mg qd), atazanavir (300 mg qd), ritonavir (100 mg qd) and saquinavir (2000 mg qd). Prior to liver transplantation, serum trough concentrations of protease inhibitors (C_{min}) were 1.22 µg/ml for atazanavir (expected range: 0.15-0.18 µg/ml), 6.13 µg/ml for ritonavir (expected level: 2.1 µg/ml) and 3.4 µg/ml for saquinavir (expected range: 0.1-0.25 µg/ml). No signs or symptoms of protease inhibitor over-dosage were observed. After questioning, the patient reported the use of a cat's claw (*Uncaria tomentosa*) preparation during the last 2 months. 15 days after withdrawal the preparation, the C_{min} values had normalised to 0.3 µg/ml for atazanavir, 0.92 µg/ml for ritonavir and 0.64 µg/ml for saquinavir. There is known detail on the herbal preparation, used by the patient (Lopez Galera *et al.* 2008).

Assessor's comment: There are no data in the article about the cat's claw preparation.

American Herbal Products Association has given *Uncaria tomentosa* interaction class A (no clinically relevant interactions are to be expected) (Gardner and McGuffin, 2013). According to the American Hospital Formulary Service classification system, the level of evidence rating for potential drug interactions is D or poor (Gardner and McGuffin, 2013).

5.5.5. Fertility, pregnancy and lactation

Reportedly, cat's claw has also been used as a contraceptive by several different tribes of Peru but only in very large dosages. The Asháninka boil 5 to 6 kg (about 12 pounds) of the root in water until it is reduced to little more than 1 cup. This decoction is then taken 1 cup daily during the period of

menstruation for three consecutive months; this supposedly causes sterility for three to four years (Rain-tree 2004).

American Herbal Products Association has given *Uncaria tomentosa* a class-2b safety rating (not to be used during pregnancy) (Gardner and McGuffin, 2013).

Safety during pregnancy and lactation has not been established.

No fertility data available.

5.5.6. Overdose

No data available

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No data available

5.5.8. Safety in other special situations

Not applicable

5.6. Overall conclusions on clinical safety

Only few and mild side effects are reported for herbal preparations of *Uncaria tomentosa*.

There is only one case report, concerning interactions, the co-administration of *Uncaria tomentosa* with medications, that substrates of isoenzyme CYP3A4 may not be beneficial.

Safety during pregnancy and lactation has not been established.

The use in children and adolescents under 18 years of age has not been documented.

6. Overall conclusions (benefit-risk assessment)

The Ashaninka Peruvian aboriginals have used cat's claw (*Uncaria tomentosa*) against several conditions, such as inflammation, cancer, gastric ulcer or as a contraceptive, emmenagogue.

Documented scientific evidence from *in vitro* and to a lesser extent, animal studies provide some supportive evidence for some of the uses of Uncariae tomentosae cortex. The preclinical pharmacological data is quite diverse on *Uncaria tomentosa*, concerning the used extracts and biological properties tested. The main concern is, that most of the studies indicate 'bark' for the drug used, without specifying whether it is the root bark or the stem bark. From the 35 studies in the primary pharmacodynamics section 19 studies mentioned just 'bark', 7 studies didn't state the part used and only 5 and 3 studies specified stem bark and root bark, respectively. A number of investigations used commercial products (food-supplement), without adequate information on the contained drug/extract.

Considering the small sample size of the human clinical studies and that the 12 conducted trials investigated 7 different effects of *Uncaria tomentosa*, using different extracts, more trials are needed in order to allow a firm conclusion on clinical efficacy of herbal preparations of *Uncaria tomentosa.* Based on the clinical evidence, the well-established use of Uncariae tomentosae cortex is not acceptable in any of the investigated conditions.

There are two countries in the European Union where products containing *Uncaria tomentosa* (Willd.) DC, cortex have been used traditionally for medicinal purposes:

In Spain preparations containing powdered stem bark of *Uncaria tomentosa* have been in medicinal use since 1998. In Hungary the powdered stem bark of *Uncaria tomentosa* and an ethanolic extract have been marketed since 2000 and 2001. Although preparations containing powdered stem bark of *Uncaria tomentosa* have been in medicinal use for a period of at least 15 years in the European Union (in Spain since 1998), evidence for the medicinal use for at least 30 years outside of the European Union of the plant part used, the specified pharmaceutical formulation, indication and posology is not available as requested by Directive 2004/24/EC. Consequently a European Union herbal monograph on *Uncaria tomentosa* (Willd.) DC, cortex cannot be established at present.

Annex

List of references